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The Impact of Psychological Interventions on Wound Healing

Hayley Maree Robinson

Abstract

Wound healing is a complex biological process consisting of a number of overlapping phases. Research to date has found that psychological factors such as chronic stress and negative affective states can slow the wound healing process. The implications of these findings indicate that psychological interventions may help promote healing. However, at present only a limited amount of research has looked at the positive impact of psychological interventions in the context of wound healing. The aim of this thesis was to address some of the gaps in this research and build on the existing findings in this area. The main aims of this thesis were: 1) to review existing research on psychological interventions and wound healing; 2) to conduct experimental studies to address gaps identified in the review. These were namely to investigate whether the timing of interventions impacted the wound healing process and to further investigate social support interventions using a different intervention type.

First a systematic review was conducted looking at previous studies that investigated the effects of psychological interventions on wound healing. The results of this review highlighted areas in which future research should be undertaken and led to three experimental studies conducted using three different interventions. Timing was particularly important for clinical translation. The first study (Study 1) showed that a relaxation intervention improved skin barrier recovery after tape stripping regardless of whether it was performed before or after the injury. It also identified that pain and sleep are important in wound healing. The second study (Study 2) found that an expressive writing intervention increased the speed of re-epithelialisation of a 4mm punch biopsy when performed prior to wounding. If performed after wounding the effects on healing depended on the timing of the writing. The results suggest that changes in affect as a result of the expressive writing may influence the speed of healing. Immunohistochemical analyses of the healing tissue two weeks later supported the results showing that expressive writing lead to increased Langerhans cells in the dermis. The final study (Study 3) found that participants who completed a social closeness task and
underwent a tape stripping procedure with another person had faster skin barrier recovery compared to people who went through the tape stripping procedure alone. Saliva analyses indicated that being with another person buffered autonomic activity over the tape stripping period.

Taken together, these studies support previous research that psychological interventions can help promote healing. The experimental studies offer several novel findings, including the importance of the timing of psychological interventions for wound healing. In addition, the thesis provides the strongest evidence to date that social support can improve healing in human participants. These studies provide insight into the complexities of the wound healing process and how psychological interventions could be implemented in clinical settings. Future research is needed in clinical settings to see if the results generalise to more extensive surgical wounds or chronic ulcers.
Acknowledgements

There are a number of people who deserve recognition for the support that they have offered, without which this thesis would not have been possible. Firstly, I would need to express my gratitude to Liz Broadbent, whose patient, dedicated, calm and good natured personality has seen me through the last few years and I can only hope has rubbed off on me. Without her guidance this thesis would not have been completed. I am also indebted to Paul Jarrett, who approached all the research in this thesis with enthusiasm that never wavered and was always encouraging and supportive.

I need to thank everyone who took part in my research. The nature of my topic meant that you had to endure red arms and a degree of pain and despite this a great number of my friends and colleagues volunteered themselves. Your small scars are a testament to this work. There are also those that helped design the research protocols and run these studies. A special thanks goes to those who collaborated on this research, John Tarlton, Christine Whiting, Kavita Vedhara, Urs Nater, Nadine Skoluda, Sam Norton and Abhimati Ravikulan. To the staff at the Clinical Research centre for accommodating me over all that time your support made running these projects so much easier.

To my friends and family, who have been with me for this entire journey, even if you didn’t completely understand what was going on in my world you knew that this process was not an easy one and supported me no matter what. To Amy, Annie, Justinn, Kate and Ali, we were stuck in the windowless refrigerator together but at least we were all in it together. It was nice to know that there were others that were enduring the trials as I was.

Finally, the biggest thank you to Josh, moving to Auckland with you is a decision we made together and I have never regretted it. Over this time you have given me the confidence to keep going when problems arouse, supported me when they seemed overwhelming and celebrated the highs with me. You have always been there for me and I know whatever comes next we will do it together.
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Chapter 3: The effects of psychological interventions on wound healing: A systematic review of randomized trials

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<td>Dr. Sam Norton</td>
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<td>Dr. Paul Jarrett</td>
<td>Study conception and editing of final manuscript</td>
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Chapter 4: The effects of relaxation before or after skin damage on skin barrier recovery: A preliminary study


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Chapter 6: The effects of expressive writing before or after punch biopsy on wound healing

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Chapter 7: Expressive writing influences wound healing: Preliminary immunohistochemistry analysis of skin tissue two weeks after punch biopsy wounding


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<td>Contribution to study design and editing of final manuscript</td>
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<tr>
<td>Prof. John Tarlton</td>
<td>Tissue sample analysis and editing of final manuscript</td>
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<tr>
<td>Dr. Christine Whiting</td>
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<td>28th October</td>
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<td>Prof. John Tarlton</td>
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<td>4th November 2016</td>
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**Chapter 7: The role of social closeness during tape stripping to facilitate skin barrier recovery: Preliminary findings**

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<td>Abhimati Ravikulan</td>
<td>Data collection and data entry</td>
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<tr>
<td>Dr. Urs Nater</td>
<td>Contribution to study design, analysis of salivary samples and editing of final manuscript</td>
</tr>
<tr>
<td>Nadine Skoluda</td>
<td>Contribution to study design, analysis of salivary samples and editing of final manuscript</td>
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<tr>
<td>Dr. Paul Jarrett</td>
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<td>AVI</td>
<td>Affect Valuation Index</td>
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<tr>
<td>ANOVA</td>
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<td>ANCOVA</td>
<td>Analysis of Covariance</td>
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<tr>
<td>AUC&lt;sub&gt;i&lt;/sub&gt;</td>
<td>Area under the curve with respect to Increase</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<td>CD</td>
<td>Cluster of Differentiation</td>
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<td>CI</td>
<td>Confidence Interval</td>
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<td>CONSORT</td>
<td>Consolidated Standards of Reporting Trials</td>
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<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<td>HLA</td>
<td>Human Leukocyte Antigen</td>
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<td>LIWC</td>
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<td>PA</td>
<td>Positive Affect</td>
</tr>
<tr>
<td>PRISMA</td>
<td>Preferred Reporting Items for Systematic Reviews and Meta-Analyses</td>
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<tr>
<td>PSS</td>
<td>Perceived Stress Scale</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<td>PSQI</td>
<td>Pittsburg Sleep Quality Index</td>
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<td>RCIT</td>
<td>Relationship Closeness Induction Task</td>
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<td>RCT</td>
<td>Randomised Controlled Trial</td>
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<td>SE</td>
<td>Standard Error</td>
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<td>SAM</td>
<td>Sympathetic Adrenal Medullary</td>
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<td>SMD</td>
<td>Standard Mean Difference</td>
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<td>TEWL</td>
<td>Transepidermal Water Loss</td>
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<td>UAHPEC</td>
<td>University of Auckland Human Participants Ethics Committee</td>
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<td>VAS</td>
<td>Visual Analogue Scale</td>
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Chapter 1: Overview

Research stemming from the emergence of the field ‘psychoneuroimmunology’ has highlighted the role of stress in health, healing and immunity (Godbout & Glaser, 2006). Psychoneuroimmunology specifically focuses on the interaction between psychological, immunological and physiological components to give a holistic understanding of health (McCain, Gray, Walter & Robins, 2005; Robins et al., 2013; Starkweather, Witek-Janusek & Mathews, 2005). To illustrate these interactions, research shows that emotional responses can have direct effects on stress hormones (Kiecolt-Glaser, McGuire, Robles & Glaser, 2002), which in turn modulate the immune response (O’Leary, 1990), essential for fast wound healing (Park & Barbul, 2004). Although there is now a large amount of evidence implicating stress in poor healing (Walburn, Vedhara, Hankins, Rixon & Weinman, 2009), research examining ways to reduce stress and improve healing using psychological techniques has received less attention. In light of this, the overarching aim of the current thesis was to evaluate existing research that has used psychological interventions to improve wound healing and to build on this body of research. In the following chapters, research looking at the role of psychology in wound healing is assessed, the rationale for looking at various psychological interventions to promote wound healing is proposed, and a series of empirical studies are presented. A brief structural overview of the thesis, and how each chapter relates to this overall aim, is outlined below.

To build a foundation for the current body of work, this thesis begins in Chapter 2 by introducing the literature on psychological stress and wound healing. This chapter briefly describes the complexities of the wound healing process and how the body’s biological response to psychological stress can affect the stages of healing. In order to lay the groundwork showing the link between psychology and healing, relevant studies that investigate the effects of psychological stress on immunology and wound healing are reviewed. To understand the breadth of research that has been conducted in various populations, the literature has been categorized according to wound type
to demonstrate the differences in methodology and assessment. This chapter also reviews other negative psychological states and their links to poor immunity and healing via similar biological mechanisms. This chapter introduces the notion that psychological interventions that are known to reduce stress may have value in promoting wound healing.

In Chapter 3 the scope of this thesis is narrowed to focus specifically on psychological interventions that may help promote wound healing. This chapter presents a systematic review (Robinson, Jarrett, Norton & Broadbent, in press) of published studies that have been conducted using a marker of healing as an outcome. This is the first systematic review to look at a number of different psychological variables and the efficacy of such interventions to improve wound healing. This review highlights that although a number of psychological interventions are known to have positive effects on health outcomes, comparatively little research in has looked at the effect of psychological interventions on healing. The findings of this review illustrate that relaxation interventions have been conducted with a variety of different populations, both within clinical and experimental settings, and although promising, is an area where more research is needed. Expressive writing is a intervention that may be of benefit but so far only two studies have been performed with writing prior to healing, and none with writing after healing. Similarly, research on social support interventions has showed encouraging findings in animals, but few studies have been conducted in humans. Other interventions identified in this review were hypnosis, yoga, cognitive behavioural therapy, stress management therapy and placebo effects, all of which indicate that more extensive research needs to be conducted in this area to determine which interventions are appropriate and applicable to patients to improve healing. However, before more clinical studies can be conducted, there is a need for more experimental research in the laboratory to establish which interventions may have the most value and how they could be implemented in healthy populations where wounds can be standardised and assessed uniformly. Another important research gap identified as a result of this systematic review was a logistical question around the timing of psychological interventions and when they should be implemented (before or after wounding) to
have the best effects on healing. This question is subsequently considered throughout the rest of this thesis in experimental settings. Previous work has not manipulated the timing of such interventions, even though this could have implications for surgical patients in terms of their care prior to and post-surgery.

After noting the benefits of psychological interventions and the need for more research in this area, Chapter 4 presents the first experimental study in the thesis (Study 1; Robinson, Jarrett & Broadbent, 2015). In line with the finding that research has neglected to address the timing of psychological interventions, this study aimed to investigate whether relaxation could improve healing regardless of whether it was delivered before or after wounding. In this study, a tape stripping procedure was conducted on healthy human participants and skin barrier recovery after tape stripping was measured as a way of measuring wound healing. The tape stripping model provides an easy, non-invasive way of measuring healing over a brief period of time. Participants were randomly allocated to either a control group (who did not receive the relaxation intervention), or to perform the relaxation intervention immediately before or after tape stripping. The results found that the relaxation intervention promoted skin barrier recovery regardless of when it was performed and also reduced perceived stress and pain.

A subsidiary finding from Study 1 was that sleep impacted skin barrier recovery. As described in Chapter 2, a number of health behaviours can impact wound healing and lack of sleep can impair healing but may also reflect high levels of stress, anxiety or depression. Chapter 5 reports the impact of sleep in relation to skin barrier recovery (Robinson, Jarrett & Broadbent, in submission). This research adds to previous work by showing that males and females who reported more sleep had better skin barrier recovery. The results are similar to previous research conducted with female samples only. This finding is discussed in terms of the impact sleep has on the immune system, in particular the relationship between lack of sleep, the release of stress hormones and healing.
Study 1 presented in Chapter 4 was conducted with healthy participants who received a tape stripping wound. As this wound involves minor partial epidermal loss, healing occurs over a short period of time. Chapter 6 presents the second experimental study of this thesis using a punch biopsy wound, which was assessed over two weeks (Study 2; Robinson, Jarrett, Vedhara & Broadbent, 2017). This study is again a randomised controlled trial but focused on a different psychological intervention, expressive writing. Chapter 3 identified that two other studies have looked at expressive writing and wound healing with this type wound. The research therefore builds on the findings of previous research but also, similar to Study 1, addressed the question of the timing of the intervention. Participants were randomised to perform expressive writing or control writing before or after the 4mm punch biopsy wound, which was taken from the inner arm and photographed 10 days and 14 days later to assess healing. A further aim was to examine whether the intervention caused changes in mood over the healing period in order to understand how psychological factors influence healing. The results found that participants who performed expressive writing before wound healing had better healing than people who performed control writing. However, those who performed expressive writing after the biopsy did not show improvements in healing in comparison to control groups. Interestingly, those that performed expressive writing immediately after being wounded, and completed the writing intervention within the first 6 days had better healing than people who started the intervention later. This suggests that the timing of the intervention is important, especially because the analysis showed that expressive writing causes short-term negative effects on mood before positive effects emerge. It is suggested that writing should therefore occur before or as close to wounding as possible so negative effects have time to dissipate. This study highlights the need for more research in this area looking not only at the effects of expressive writing on psychological factors, but how psychological changes at different stages of the healing process may influence healing.

Having demonstrated that expressive writing can positively influence wound healing, particularly if it is conducted prior to wounding in Chapter 6, Chapter 7 reports the findings of the
immunohistochemical analyses from this Study 2 (Robinson, Jarrett, Vedhara, Tarlton, Whiting & Broadbent, in preparation). In addition to taking the original biopsy and assessing healing over two weeks by photographs, a second larger biopsy, incorporating the first biopsy, was taken two weeks after the original biopsy in order to investigate immunological differences within the tissue between participants that performed the expressive writing intervention and the control writing task before and after wounding. The findings reported are preliminary as tissue sample analysis is ongoing, but provide support for the findings in Chapter 6. The results showed that participants in both expressive writing groups had significantly higher number of Langerhans cells than the control groups. Higher numbers of Langerhans cells was associated with fewer neutrophils and macrophages. Furthermore fewer neutrophils and macrophages were associated with faster healing. This data gives insight into the immunohistochemical changes that occur within the skin tissue that are predictive of faster healing. Although more research is needed linking immunohistochemical markers and psychological factors to confirm these findings, this research shows that psychological interventions can affect wound healing processes at a cellular level.

In Chapter 8 the last experimental study of the thesis is detailed (Study 3; Robinson, Ravikulan, Jarrett, Skoulda, Nater & Broadbent, 2017). This experimental research is again guided by the systematic review conducted in Chapter 3, which identified social support interventions as an area needing further research. The systematic review in Chapter 3 demonstrated that the research conducted to date has been done with a variety of population types, at different timepoints, with different ways of providing social support (peer support groups versus formal support before surgery versus support before a stressor task). Hence more research is needed to understand how social support may be helpful and how it could be applied in a clinical setting. Study 3 was designed to mirror a clinical setting (e.g. a hospital ward or waiting area) by investigating whether social closeness elicited from another stranger undergoing the same tape stripping procedure could help reduce stress and improve healing. A randomised trial was conducted in which participants were randomised either to undergo tape stripping in pairs or alone. The participants completed a task to
foster social closeness and then remained in the same room throughout the procedure. Skin barrier recovery was measured 30 minutes after tape stripping and compared to participants in the control condition where they went through the tape stripping procedure alone. The results showed that participants who had undergone the social closeness task and the tape stripping procedure together had faster skin barrier recovery in comparison to the control participants. Furthermore, over the course of the study session saliva samples were obtained throughout the experimental procedure to determine if the benefits of social support were related to decreases in autonomic arousal and stress hormones. The results showed people in the social closeness condition had lower alpha-amylase reactivity after tape stripping and indicate that a social closeness intervention can reduce autonomic arousal and improve wound healing.

Finally Chapter 9 summarizes the main findings of the thesis and ties them together in the discussion. The research conducted in this thesis builds on previous research investigating the beneficial effects of psychological interventions on wound healing. Together, this body of research shows the importance of timing the implementation of such interventions and demonstrates in detail that psychological interventions impact biological mechanisms involved in healing, although more research is required to understand the complexities of psychological influences on the wound healing process. Study 1 provided evidence that relaxation is beneficial either before or after wounding, Study 2 indicates that expressive writing needs to be performed prior to wounding, or if not, as close to wounding as possible, and Study 3 found that social closeness from another person undergoing the same procedure can also aid healing. These results are discussed in the context of the broader literature on psychology and wound healing as well as clinical implications. The research limitations are acknowledged and the thesis concludes with a discussion of future research possibilities and directions.
Chapter 2: Introduction: Psychological factors and wound healing

Overview

This chapter reviews previous research examining the relationship between psychological stress and wound healing. The biological stress response, its links to immunity and subsequent wound healing are briefly outlined. Then key evidence from the studies on stress in experimental and clinical wounds is described. The other psychological and physical factors that can influence the stress response are also outlined and these include depression, anger, social isolation, optimism, pain, sleep and health behaviours. The number of consistent results showing the effects of psychological factors on wound healing suggests that psychological interventions to reduce or buffer the stress response could be beneficial.

The stress response

A stress response occurs when events or environmental demands exceed an individual’s perceived ability to cope (Lazarus & Folkman, 1984). Environmental stressors can elicit heightened arousal in order to deal with the threat. However, if the threat is not dealt with immediately the autonomic nervous system remains on high alert and this long-term stress results in immunosuppressive effects on the body (Williams & Leaper, 1998).

Stress activates the sympathetic nervous system, triggering the fight-or-flight response. As a result the hypothalamic–pituitary–adrenal (HPA) and sympathetic–adrenal–medullary (SAM) axes both release stress hormones into the body. The SAM axes allows the release of epinephrine and norepinephrine (known as catecholamines) into the bloodstream, preparing the body for fight-or-flight by signalling physiological changes (increased heart rate, blood pressure, respiration, sweating). Once the initial surge of catecholamines has subsided, the HPA axis takes over, releasing other stress hormones, such as cortisol, a glucocorticoid that signals the sympathetic nervous system to stay alert. Once the stressor has gone the parasympathetic takes control over the body to restore balance and dampen the stress response.
Impact of stress hormones on the immune system and healing

The wound healing process is a complicated sequence of events, reliant on each aspect of the immune system functioning well at each stage of healing to prevent prolonged inflammation. Wound healing is an important marker of overall health and immunity in healthy populations (Guo & DiPietro, 2010). Wound healing is a complex process where the normal tissue structure or function is disrupted, triggering a number of changes in the body to facilitate healing. This process starts immediately and can be divided into a number of overlapping phases; inflammation, proliferation and wound remodelling (Gosain & DiPietro, 2004; Thompson, Chang & Jobe, 2006). During the initial inflammatory stage, vasoconstriction and blood coagulation are followed by the activation of platelets, which release growth factors (van de Kerkhof, van Bergen, Spruijt & Kuiper, 1994). These attract phagocytes (neutrophils and monocytes) to the site that eliminate bacteria and phagocytose debris, preparing the wound site for new tissue growth. In the proliferative phase, recruitment and replication of cells necessary for tissue regeneration and capillary regrowth begin. In the final phase, collagen fibres are formed and then remodelled forming permanent scar tissue (Martin, 1997). This phase may last for weeks and months (Glaser & Kiecolt-Glaser, 2005). The length of time during each phase and success in the later stages of wound repair is highly dependent on the initial immune response (Hübner et al., 1996). In clinical settings where surgical wounds are created or wounds are treated, faster healing can reduce costs associated with care and recovery (Sen et al., 2009).

Optimal healing of wounded tissue involves a delicate trade-off between inflammation and rapid wound closure (Martin & Leibovich, 2005; Szpaderska & DiPietro, 2005). The release of cortisol can moderate inflammation that occurs after injury, by decreasing cytokine production at the wound site. If stress is ongoing, the continued release of cortisol can result in a prolonged inflammatory reaction that can delay wound healing in later stages (Engeland, Bosch, Cacioppo & Marucha, 2006). Elevated cortisol levels impair wound healing by contracting blood vessels. This alters the amount of oxygen delivered to the wound bed and can negatively influence tissue oxygenation, cell proliferation, collagen synthesis, and infection prevention (Rodriguez, Felix, Woodley, & Shim, 2008).
Furthermore, cortisol has been shown to reduce macrophage and neutrophil phagocytosis, which compromised bacterial proliferation and clearance in the wound bed (Palermo-Neto, de Oliveira Massoco, & Robespierre de Souza, 2003; Rojas, Padgett, Sheridan, & Marucha, 2002). This may lead to a higher chance of wound infection (Robson, 1997; Rojas et al., 2002).

Research with animal models also shows the detrimental effects of stress on healing (Sheridan, Padgett, Avitsur & Marucha, 2004; Romana-Souza et al., 2010). For example, treatment with dexamethasone (a synthetic glucocorticoid) delays wound healing (Hubner et al., 1996). Furthermore, research with hamsters has found that removal of endogenous corticosteroids via adrenalectomy helps to prevent stress-related delays in wound healing but has no effect on the rates of healing if the animal is not subjected to stress (Detillion, Craft, Glasper, Prendergast & DeVries, 2004). In line with this finding research has also shown that glucocorticoid antagonists can partially restore the effects of restraint stress on wound healing, wound cytokine production, and wound infection (Head, Farrow, Sheridan & Padgett, 2006; Padgett, Marucha & Sheridan, 1998; Rojas, et al., 2002).

**Experimental research examining the link between stress and wound healing**

Over recent decades research has demonstrated the negative effects of stress on the immune system in relation to wound healing. Walburn and colleagues (2009) reviewed research in humans exposed to clinical stressors (surgery), naturalistic life stress (examinations, marital dissolution, general life stress) or experimentally induced stress (interview) and evaluated the affects on healing in a variety of wound types. Of the 22 papers included in the systematic review, 11 papers were included in a meta-analysis. They found a pooled moderate effect size of $r = .37$, indicating that psychological stress has a moderately sized negative influence on wound healing.

**Methods of assessing wound healing**

Studies on the influence of stress on wound healing have used a variety of different methods. Wound healing has been assessed in experimental studies using standardised wounding
methods such as punch biopsies, blister creation and tape stripping (Koschwanez & Broadbent, 2011). In clinical settings, studies have been conducted with patients who have chronic ulcers and surgical wounds. Methods of analysing wound healing have included not only at the time it takes for wound closure or re-epithelialisation to occur, but also looking at healing wound tissue and wound fluid to examine immune markers associated with healing. Despite the number of ways wounds have been created and healing has been measured, research has consistently shown that stress negatively influences healing. Briefly, the most influential papers illustrating this relationship using various wound healing models are described below.

**Punch biopsy wounds**

One of the first studies looking at the relationship between wound healing and stress was conducted by Kiecolt-Glaser and colleagues (1995). In their research 13 caregivers of a loved one with Alzheimer’s disease were compared with 13 matched controls. All participants received a 3.5mm punch biopsy wound to the upper arm, which were monitored for 8 weeks. During the healing period hydrogen peroxide was applied to the wound and the wound was deemed fully healed when there was no foaming response when hydrogen peroxide was applied. The results showed that caregivers were significantly more stressed and took significantly longer to heal than the controls by 10 days.

Another study that is well cited because it clearly demonstrates that periods of stress are associated with slower wound healing was conducted by Marucha et al. (1998). Eleven dental students received a 3.5mm punch biopsy wound to the hard palate in the mouth, once during summer vacation and once again during exam time. Again healing was assessed using foaming hydrogen peroxide. The results found that on average it took 3 extra days for the wound to heal during the exam compared to summer vacation. Production of the cytokine Interleukin-1 (IL-1) in the blood was lower in all students during exam time compared to vacation. IL-1 is a pro-inflammatory
cytokine involved in attracting phagocytes and lymphocytes to the wound site and hence a lower response would impact speed of healing.

Research shows that wound healing can be delayed based on an individual’s general levels of perceived stress (Ebrecht et al., 2004). Twenty-four healthy males who participated in this study each received a 4mm punch biopsy wound. The wound was monitored using high-resolution ultrasonic scanning of the base of the wound and participants were asked to complete a perceived stress scale and provide a cortisol sample 2 weeks before the biopsy, on the day of the biopsy and 2 weeks after the biopsy. The results showed that people who reported higher stress had significantly slower healing. In addition elevated cortisol was negatively correlated with wound healing. When the sample was split into fast and slow healing rates, those that displayed slower healing had higher perceived stress and higher cortisol levels.

**Blister wound model**

Research using other methods of wound creation has found similar results to biopsy studies. For example, the blister wound model creates a blister by applying vacuumed pressure to the skin (normally on the forearm; Glaser et al., 1999). Research using this method found that cortisol levels at the time of wounding were inversely associated with inflammatory cytokines and metalloproteinase production in the blister wound (Glaser, et al., 1999; Yang, Bane, MacCallum, Kiecolt-Glaser, Malarkey & Glaser, 2002).

**Tape stripping model**

The tape stripping model is another way of experimentally inducing wounds. It involves removing the outermost layer of keratinocytes (stratum corneum) by repeatedly applying and removing cellotape to an area of skin (normally of the inner forearm; Altemus, Rao, Dhabhar, Ding, & Granstein, 2001; Nickoloff & Naidu, 1994; Robles, 2007). The skin is a self-renewing organ made up of several layers. The main function of the skin is to serve as a protective barrier against the outside world. It is an integral part of the immune system, protecting the body against foreign pathogens.
and preventing fluid loss. Any injury to the skin must be rapidly and efficiently mended. The skin has three layers; the bottom layer (consisting of subcutaneous fat and connective tissue), a middle layer, (the dermis) and the top layer (the epidermis). The epidermis is commonly divided into four layers and the outermost layer is called the stratum corneum, which is composed of protein, lipid cells and dead keratinocytes, termed corneocytes. Any changes to the composition of the stratum corneum results in altered barrier function (Baroni, et al., 2012). The dermis functions to continuously renew the epidermis. However, only 15% of cells are involved in this constant process, while the remaining cells are in a resting state and are only active when enhanced proliferation is required, such as the healing process (Blanpain & Fuchs, 2006).

Damage to the stratum corneum alters skin barrier function as measured by transepidermal water loss (TEWL). To measure TEWL the water vapour gradient in the air layer adjacent to the skin is assessed using a probe pressed against the skin. With higher skin damage more water loss can be expected and as the skin heals the vapour loss diminishes (Altemus et al., 2001; Cua, Wilhelm, & Maibach, 1990; Muizzuddin, Matsui, Marenus, & Maes, 2003). Research has again demonstrated that higher perceived stress can influence skin healing, as measured by the percentage the skin barrier function has recovered after tape stripping (Garg et al., 2001; Muizzuddin et al., 2003).

Altemus et al. (2001) investigated the effect of three different type of stress (psychological stress, sleep deprivation and exercise) on skin barrier function in a group of healthy women. The findings indicated that skin barrier recovery was impaired after the interview stressor and after sleep deprivation, in comparison to pre-test. Furthermore, cytokine responses to the interview stressor were correlated skin barrier function recovery, indicating that higher levels of cytokines impaired healing. In another study conducted by Garg et al. (2001), skin barrier recovery was measured in a sample of healthy students at three different time points. Skin barrier function was measured during examinations as well as during after winter vacation and during spring vacation. The results found that during the exam period students reported higher levels of stress compared to vacation and this
was indicative of worse skin barrier recovery. Furthermore, students who reported high levels of stress overall had poorer healing. Lastly, skin barrier recovery was assessed in a group of women who were engaged in marital dissolution (Muizzuddin et al., 2003). It was hypothesized that these women would have higher levels of stress and well as slower skin barrier recovery in comparison to an age-matched control group. The findings supported the tape stripping studies showing that higher levels of stress were associated with poorer skin barrier recovery.

The findings of the studies conducted with human participants have been supported in animal research. Research with mice shows that stressor such as crowded living conditions, immobilisation and transfer to a different environment can inhibit skin barrier recovery (Denda, Tsuchiya, Elias, & Feingold, 2000; Denda, Tsuchiya, Hosoi & Koyama, 1998).

**Wound healing in clinical settings**

Research has been conducted not just in experimental settings but also in clinical settings with post-surgical patients and patients with chronic wounds. These studies use slightly different methods to assess healing because unlike the experimental wounds these wounds are not standardised, but the findings still show the impact of stress on wound healing.

**Chronic wounds**

Although there is little research looking at stress and healing in patients with chronic wounds, research conducted with patients with chronic leg ulcers has found that patients with higher scores in depression and anxiety had delayed healing (Monami et al., 2008; Vedhara et al., 2010). Assessment of healing for chronic wounds usually involves calculating the percentage of the wound that has healed or rating the healing of the wound using a standardised scale. Research has found that patients with higher anxiety and depression scores were four times more likely to have delayed healing when assessed 3 months later (Cole-King & Harding, 2001). People with chronic wounds such as leg ulcers often experience reduced functionality, quality of life and social isolation.
and therefore, stress, depression and anxiety are commonly reported (Jones, Barr, Robinson & Carlisle, 2006; Valencia, Falabella, Kirsner & Eaglstein, 2001).

**Surgical wounds**

Surgery can be a very stressful time for patients and research has found that greater anxiety and distress is associated with poor recovery rates and higher instances of complications (Johnston & Wallace, 1990; Mathews & Ridgeway, 1981). As expected, surgery causes elevations in glucocorticoids due to increased HPA activity (Salomaki, Leppaluoto, Laitinen, Vuolteenaho, & Nuutinen, 1993), which in turn can have negative effects on the immune system (Pollock, Lotzova, & Stanford, 1991). Broadbent and colleagues (2003) explored the effect of psychological stress on wound repair after surgery for inguinal hernia in 47 patients. Wound fluid was collected from the wound site and analysed for IL-1, IL-6 and matrix metalloproteinase-9. The results showed that greater pre-operative stress was significantly linked to lower levels of IL-1. Greater worry about the operation was significantly associated with lower levels of matrix metalloproteinase-9, higher pain and slower recovery. This shows that in a clinical environment psychological stress impairs the inflammatory response and matrix degradation in the wound immediately following surgery.

Another study (Maple et al., 2015) looked at the impact of stress on 52 kidney donors due to undergo a hand-assisted laparoscopic donor nephrectomy. Patients completed a number of psychological measures prior to surgery, including a measure of perceived stress, and healing of the surgical wound was analysed during the recovery period with a high-resolution ultrasound scanner. The results found that higher pre-operative life stress were associated with delayed wound healing as shown by wound width and amount of tissue fluid measured from post-operative scans.

**Skin conditions**

Finally, research suggests that stress not only has a role in wound healing but also in the exacerbation of skin diseases such as psoriasis and atopic dermatitis (Al’Abadie, Kent & Gawkrodger, 1994; Locala, 2009). Research has found that increased glucocorticoid production following stress or
administration of system glucocorticoids adversely influenced skin barrier function in these patients (Orion & Wolf, 2012).

**Stress and wound healing summary of literature**

Overall the literature indicates consistent negative effects of stress on wound healing. However, due to the complex nature of the wound healing process, and the complex effects that stress has on the immune and endocrine systems, the mechanisms underlying the effects of stress on wound healing are not understood completely. The next section highlights other psychosocial factors that can influence the biological stress response and alter the neuroendocrine pathways that are activated during stress.

**Other psychological factors influencing wound healing**

Changes in mood and emotional responses can also cause neuroendocrine changes in the body and may operate via similar pathways to stress, resulting in altered immune responses. It is important to note that there are other factors that can activate the HPA and SAM axis, releasing stress hormones into the body and influencing immune function and wound healing.

**Depression**

During depression neuroendocrine changes, such as elevated cortisol levels, lead to adverse immunological function (Herbert & Cohen, 1993). Research has found that major depression is related to higher pro-inflammatory cytokine levels, including IL-6 (Miller, Stetler, Carney, Freedland, & Banks, 2002; Glaser, Robles, Sheridan, Malarkey, & Kiecolt-Glaser, 2003) and higher levels of depression are linked with increased IL-6 levels two weeks after receiving an influenza virus vaccination challenge (Glaser et al., 2003; Pace et al., 2006). Conversely, treatment with antidepressant medications reduces pro-inflammatory levels (Kenis & Maes, 2002). Findings from further research both with animal models and humans indicate that the relationship between inflammation patterns and depression is bi-directional, as administration of pro-inflammatory cytokines can induce depressive symptoms. For example, when mice receive a dose of pro-
inflammatory cytokines sickness behaviour is observed, which resembles depressive symptoms in humans (Dantzer O’Connor, Freund, Johnson & Kelley, 2008). In humans, following the administration of cytokines, mood worsens and pro-inflammatory cytokine production increases (Raison, Capuron & Miller, 2006). Further research has revealed that the resulting low mood is associated with reduced connectivity in the brain areas implicated in depression, a mechanism modulated by peripheral IL-6 (Harrison et al., 2009).

Depression can influence a number of surgical outcomes including length of hospital stay, return to daily activities, pain reports, analgesic use, and likelihood of complications, including death (Blumenthal et al., 2003; Kiecolt-Glaser, Page, Marucha, MacCallum & Glaser, 1998; Rosenberger, Jokl & Ickovics, 2006). Research indicates that depression can impact wound healing as well. For example, a study looking at the impact of depression on wound healing was conducted with otherwise healthy people who scored in the upper or lower quartiles for depression of the initial sample (Bosch, Engeland, Cacioppo & Marucha, 2007). All the participants received a 3.5mm punch biopsy wound to the oral hard palate. Highly dysphoric participants had slower healing compared to the rest of the sample after controlling for demographic and behavioural variables. More depressed participants also exhibited significantly larger average wound sizes from day 2 post wounding onwards.

As previously mentioned research has identified depression as a factor that was associated with poor foot ulcer healing (Vedhara et al., 2010). As an example, Monami et al. (2008) conducted a longitudinal study over 12 months monitoring healing and changes in foot ulcers. At 6 months higher levels of depression were linked with lower rates of healing over 6 months, whereas those with low levels of depression were more likely to have their ulcer healed. This is important since it is estimated that approximately a third of patients who present with a foot ulcer have clinical depression and depression is associated with a higher risk of mortality (Ismail, Winkley, Stahl, Chalder, & Edmonds, 2007).
Finally, one study (Wilson et al., 2011), investigated burn healing and perceptions around the injury and healing. Results indicated that depression was correlated with negative perceptions of their burn wound as measured by the Brief Illness Perception Questionnaire. Even when controlling for variables such as burn depth, burn location, demographic variables and previous psychological problems, negative perceptions about the burn still significantly predicted slower healing.

**Anger and hostility**

Anger is another emotion that might contribute to slower wound repair. Although there are a number of ways of defining anger, it commonly includes feelings that range in intensity from irritation or annoyance to intense fury and rage (Miller, Smith, Turner, Guijarro & Hallet. 1996). Anger can incorporate expression of hostility, which is defined as having a negative attitude toward others, enmity, denigration, and cynicism. Research indicates that having a hostile attitude leads to more frequent episodes of anger (Eckhardt, Norlander & Deffenbacher, 2004). Because of the way anger is expressed and experienced, there are physiological and psychological consequences (Deffenbacher, Oetting, Lynch & Morris, 1996). Anger has been linked to increased cardiovascular risk (Chida & Steptoe, 2009a) and early mortality (Harburg, Julius, Kaciroti, Gleiberman & Schork, 2003).

Anger has also been associated with changes in immune regulation. For example, caregivers of people with dementia who display high levels of anger and poor anger control had a lower mitogen immune response (Scanlan, Vitaliano, Zhang, Savage & Ochs, 2001). There is also evidence that people who have good control over anger expression show better immunity (Ishihara, Makita, Imai, Hashimoto & Nohara, 2003; Penedo et al., 2006). Furthermore, anger expression as well as depression has been associated altered with cortisol secretion (Adam, 2006; Adam, Hawkley, Kudielka & Cacioppo, 2006). During conflict in martial interactions, men who directed more anger toward their spouse, had higher levels of cortisol than men who did not express angry feelings (Miller, Dopp, Myers, Stevens & Fahey, 1999) and similar research has found that as a result of the conflict discussion more hostile couples had greater elevations in epinephrine and norepinephrine.
throughout the remainder of the day (Kiecolt-Glaser et al., 1996; Malarkey, Kiecolt-Glaser, Pearl & Glaser, 1994).

In a surgical setting research has found that people who report high levels of anger as well as anger suppression have slower post-operative recovery, and more complications (Sharma, Sharp, Walker & Monson, 2008; Stengrevics, Sirois, Schwartz, Friedman & Domar, 1996). However, only one study has looked at the impact of hostility in the context of wound healing (Kiecolt-Glaser et al., 2005). In this study couples were given a blister wound to be assessed for healing. They then took part in a discussion about a martial disagreement and a structured supportive interaction. The results found that couples who exhibited more hostile behaviours across both interactions healed 60% slower than couples that were rated as less hostile. This might be because people who express anger and hostility often experience trouble with personal relationships that repeatedly create stressful experiences (Smith & Christensen, 1992). This in turn, may precipitate exaggerated physiological reactions which translate to changes in the immune system and delayed wound healing.

Positive affect and optimism

Aside from negative emotions, positive affect and optimism have been associated with not only health benefits, but also better immunity and healing. Although optimism and positive affect are related constructs, they cannot be considered the same (Pressman & Cohen, 2005). Optimism is the generalised expectation that good things will happen where as positive affect generally reflects pleasurable engagement with the environment, and includes happiness, joy, contentment, excitement, and enthusiasm (Clark, Watson, & Leeka, 1989). Positive affect can be brief feelings or more long-lasting stable emotions.

Research has shown that positive affect is related to lower levels of morbidity, physical health symptoms and pain (Pressman & Cohen, 2005). Evidence also suggests that positive affect can influence immunity. For example, Mittwoch-Jaffe and colleagues (1995) found that after negative mood induction participants had increases in tumor necrosis factor alpha and lower levels of IL-2 and
IL-3 in the blood, consistent with heightened HPA and the sympathetic nervous system. In contrast, positive mood induction produced opposite cytokine patterns. The authors suggest that the HPA axis and the sympathetic nervous system mediate changes in the immune reactivity in response to emotions. Other research has similarly shown that positive affect, when measured throughout the day, is associated with lower cortisol levels and lower levels of circulating plasma pro-inflammatory cytokines (Steptoe, O’Donnell, Badrick, Kumari & Marmot, 2008). In line with this, positive affect has been associated with greater antibody responses to hepatitis B vaccinations (Marsland, Cohen, Rabin & Manuck, 2006) and following experimental administration of either influenza or rhino virus, decreased risk of developing illness (Cohen, Alper, Doyle, Treanor & Turner, 2006). Finally, one study has assessed the association of positive affect on wound healing. Robles and colleagues (2009) reported that skin barrier recovery was faster in participants who had higher levels of positive affect.

Research looking at optimism shows a similar pattern of results. A review of health outcomes associated with optimism (Rasmussen, Scheier & Greenhouse, 2009) found that optimism is a significant predictor of mortality rates, cardiovascular outcomes, pain, cancer outcomes and immune function. Evidence suggests that optimism can moderate the negative effect of stress on immunity. During times of stress, people with higher levels of optimism have greater levels of natural killer cells, indicating better immune responses compared to people with low levels of optimism (Segerstrom, Taylor, Kemeny & Fahey, 1998). Similarly, after receiving a vaccine, participants with high levels of optimism subject to brief stressors showed better antibody responses (Brydon, Walker, Wawrzyniak, Chart, & Steptoe, 2009). The relationship between optimism and immunity is complex and research indicates that it is dependent on the duration and type of stressor involved. As a result, not all studies report a protective effect of optimism on stress-induced immune changes (Segerstrom, 2005). Two studies have looked at the relationship between optimism and wound healing (Ebrecht et al., 2004; Maple et al., 2015). In both studies, as well as finding that higher levels of perceived stress and cortisol were related to slower healing, they also reported that lower levels of optimism were associated with poor healing. Overall, the research on
optimism and positive affect as well as other mood states suggests that individual differences in expectancies, appraisals, and mood may be important in understanding psychological and immune responses to stress.

**Social isolation and loneliness**

Social isolation and loneliness are also psychosocial factors known to influence health. Research has found social isolation is associated with increased mortality (Steptoe, Shankar, Demakakos & Wardle, 2013). Social isolation refers to the number of people within someone’s social network and the amount of contact they have with others. Loneliness is often associated with social isolation, as it is the psychological embodiment of isolation, reflecting the person’s perception or dissatisfaction with their social network (Shankar, McMunn, Banks, & Steptoe, 2011). As well as higher mortality rates, socially isolated and lonely individuals are at higher risk of cardiovascular disease (Barth, Schneider & von Känel, 2010; Thurston & Kubzansky, 2009), cognitive decline (Bassuk, Glass & Berkman, 1999), and negative psychological states such as anxiety and depression as well as negative reactivity (irritability, hostility) that all can adversely impact health outcomes (Cacioppo et al., 2002). Solitary confinement is regarded as a severe punishment in society because the need to be part of a community is adaptive for survival (Baumeister & Leary, 1995). Furthermore, ostracism, rejection or exclusion activates similar regions in the brain to physical pain (Eisenberger & Lieberman, 2004) and is more pronounced in people with low self-esteem (Onoda et al., 2010).

Perceived loneliness has been found to affect the stress response. Research shows that people who are isolated and lonely have similar stressors to others but they rate these events as more stressful (Hawkley & Cacioppo, 2010). They also may have different coping strategies, tending to use more passive coping strategies to deal with stress and may have less resources to draw on (Cacioppo & Hawkley, 2003). In line with this, people who report more loneliness have higher cortisol levels over the course of the day (Cacioppo et al., 2000; Grant, Hamer & Steptoe, 2009). On the opposite side of the scale, research shows that social connectedness can buffer autonomic
responses during stress (Cohen, 2002). Interestingly, a study conducted by Eisenberger, Taylor, Gable, Hilmert and Lieberman, (2007) found that participants who regularly had supportive interactions over a 10-day period showed lower cortisol reactivity to a stress task and this was associated with diminished activity in areas of the brain associated with emotional responses to separation. This supports research linking social support or lack thereof to negative physiological reactivity that can have detrimental influences health.

Research looking at the influence of social support has found links between immune function and perceived loneliness. This was highlighted in a meta-analysis conducted by Uchino and colleagues (1996) where several studies showed links between higher perceived levels of social connectedness, lower levels of autonomic activity and better immune responses. For example, research showed that in older people, those who had more emotional and tangible support had more natural killer cells, which play a vital role in a variety of immune functions (Esterling, Kiecolt-Glaser & Glaser, 1996). Similarly in patients with ovarian cancer, social support was associated with higher natural killer cell activity (Lutgendorf et al., 2005). Other research has found that people with little social support have worse immune responses to an influenza vaccination (Moynihan et al., 2004; Pressman et al., 2005) and that lonelier participants have higher pro-inflammatory responses when under stress (Jaremka et al., 2013). Research conducted with people under chronic stress has also found a negative impact of low levels of social support on immunity. Kiecolt-Glaser et al. (1991) reported that spousal caregivers of a person with dementia who reported low amounts of social support showed poor immune function one year later, compared to those with higher levels of social support. Hence it seems that immune dysregulation is a potential pathway via which loneliness influences health in the context of stressor.

This research indicates that lower levels of social support may have negative implications for wound healing. The physiological differences observed between people who have high and low levels of loneliness indicate modulation of the neuroendocrine and inflammatory
response to stress (Uchino, 2006). Indeed, research has found that people who report higher levels of loneliness have poor wound healing (Marucha et al., 1998). As described earlier, a study conducted by Marucha, and colleagues (1998), gave dental students a punch biopsy to the hard palate of the mouth on two separate occasions, one of high stress and one of low stress. The results found that participants healed more slowly when they were under stress. In addition, when the students were followed up 6 months later, social isolation was significantly negatively correlated with healing on both occasions. Another study conducted by Kiecolt-Glaser and colleagues (2005) found that supportive interactions were associated with a stronger acute increase in inflammatory cytokines at the site of blister wounds, resulting in faster wound healing.

Finally research with animal models supports these findings and helps delineate the mechanisms by which healing is impaired. Research with mice have found that mice isolated from others after wounding had slower wound repair compared to those that were housed with others (Glasper & DeVries, 2005; Martin, Glasper, Nelson & DeVries, 2006; Pyter, Yang, da Rocha & Engeland, 2014; Vegas et al., 2012). Detillion and colleagues (2004) found that in hamsters that were isolated and subjected to stress, cortisol concentrations increased and healing was impaired in contrast to hamsters that were not isolated. Hamsters that underwent an adrenalectomy to stop the release of endogenous cortisol during stress had similar healing rates to animals that were socially housed, implicating the role of the neuroendocrine system in a stress-buffering model. Furthermore, hamsters that were isolated but treated with oxytocin, a hormone released during social contact and associated with social bonding, had lower stress-induced increases in cortisol and better wound healing.

**Interactions between psychological and physiological factors that influence healing**

As described in the chapter so far, psychosocial factors can have a direct influence on the biological stress response and in turn affect immune functioning and wound healing. In addition, psychological factors can mediate other physiological processes that affect healing. For example,
pain and sleep not only influence wound healing directly, but also can be influenced by psychological states.

**Pain**

Pain is important to assess in research on psychology and wound healing because it is often experienced after injury. Higher levels of pain can have a negative impact on surgical recovery and are known to contribute to poor quality of life and higher levels of disability (Scudds & Robertson, 1998). When pain occurs the events that precipitate the nociceptor response are similar in most cases. Nociceptors respond to pain by sending and electrical signal to the central nervous system including information about the pain location and intensity (Widgerow & Kalaria, 2012). In response to pain signals, pain mediators are released, which include inflammatory cells that act to stimulate nociceptive neurons (Widgerow & Kalaria, 2012). Even though pain can be thought of as a personal and subjective experience and is triggered for different reasons, the response to pain in the body is the same. Normally when pain is triggered because of injury, pain mediators have a small positive impact on wound healing (Delgado, McManus & Chambers, 2005; Savla, Appel, Sporn & Waters, 2001). Pain is considered a protective physiological response because it controls and decreases inflammation. The nociceptive system can then revert back to a normal state once the underlying injury is on a healing trajectory (Dray, 1995). If pain continues or the pain response is amplified then a number of physiological responses occur that may have a direct effect on wound healing. To highlight the role that the immune system plays in pain, it is thought that pro-inflammatory cytokines may play a role in hyperalgesia (Watkins, Maier & Goehler, 1995). Other research has found that people with chronic pain have higher cortisol levels than matched controls (Van Uum, Sauvé, Fraser, Morley-Forster, Paul & Koren, 2008). It appears that the experience of pain can disrupt the expected diurnal cortisol decline, as when pain occurs cortisol increases (Al Absi, Petersen, & Wittmers, 2002). This shows how pain and wound healing involve similar pathways and the potential for increased pain to interfere with wound healing.
Many studies show advantages in outcomes by using pain relief. For example, in an animal model, rats that were given morphine pre-operatively had smaller tumours than those that were not given morphine (Page, Blakely & Ben-Eliyahu, 2001). Research shows that pain may be capable of facilitating the progression of metastatic disease highlighting the need for pain management (Page & Ben-Eliyahu, 1997).

Several factors may influence pain perceptions. For example, research has found that depression is related to greater pain intensity in chronic pain patients (Haythornwaite, Sieber & Kerns, 1991) and after knee surgery patients who reported more anxiety and depression before the surgical procedure had greater pain as well as worse outcomes one year later (Brander, Gondek, Martin & Stulberg, 2007). Other research has shown that people who are particularly anxious about the experience of pain tend to report higher levels of pain (Al Absi & Rokke, 1991). This is thought to be related in part to people’s expectations of pain. Research has found that a person’s mental representation and sensory experience of pain in the brain can influence the actual formulation of the experience. When the intensity of pain is downplayed the subjective experience of pain is less, as evidenced by activation of pain-related brain regions (Koyama, McHaffie, Laurienti & Coghill, 2005).

It should be recognised that anticipation of pain, or continued experience of pain may be considered an ongoing stressor, so wound healing may be impaired not only by the experience of pain but also the associated psychological reactions to pain. There is also some research indicating that people who were angry reported higher levels of pain (Janssen, Spinhoven & Brosschot, 2001).

Of the research that looks specifically at the impact of pain on wound healing, an association has been found between higher pain levels and wound healing. For example, McGuire et al. (2006) conducted a study with 17 women who underwent an elective bypass surgery and assessed the role of pain in wound healing. Participants were each given a punch biopsy wound after their operation and rated their post-surgical pain for 4 weeks. The results found that patients who had higher levels of acute pain and also persistent pain over 4 weeks subsequently took a longer amount of time to
heal the biopsy wound. Another study conducted by Woo and Sibbald (2009) found that pain levels reduced over 4 weeks as leg ulcers healed. Patients who achieved wound closure had significantly lower pain ratings to those that did not.

**Sleep**

Sleep is another factor that can affect wound healing. Sleep is essential for optimal health and is necessary due to its restorative effects on the body. Research has found that lack of sleep is related to overall mortality as well as cardiovascular and cancer related mortality (Gallicchio & Kalesan, 2009). Sleep deprivation has been found to affect cognitive performance and emotionality (Pilcher & Huffcutt, 1996). During sleep, processes such as muscle growth, tissue repair and protein synthesis occur and hence lack of sleep can have a detrimental effect on immune function (Besedovsky, Lange & Born, 2012). During deep sleep, growth hormones are released, which enhance multiple aspects of immune function (Veldhuis & Iranmanesh, 1996) and lack of sleep results in reductions in growth hormone secretion (Van Cauter et al., 1992). Research has also shown that after sleep deprivation natural killer cell activity is reduced (Irwin et al., 1994), sympathetic nervous system activation increases (Irwin, Thompson, Miller, Gillin & Ziegler, 1999), HPA activity increases (Leproult, Copinschi, Buxton & Van Cauter, 1997; Spiegel, Leproult, & Van Cauter, 1999) and host defence responses are altered (Benca & Quintans, 1997; Marshall & Born, 2002; Opp & Toth, 2003). Therefore, disturbances to sleep and sleep architecture result in down-regulation of tissue repair processes and immune function (Lee & Stotts, 1990).

Sleep deprivation, as well as influencing immune processes, can also be viewed as a stressor in itself. Hence the relationship between sleep, stress and immunity is complex. Sleep deprivation may have a direct effect on wound healing and it is possible that associations between distress and impaired wound healing are mediated by lack of sleep. Sleep deprivation may also affect how a person reacts to stressors. Research shows that when people have restricted sleep, they react to stressors with more emotion. For example, in medical residents, negative emotional perceptions of unforeseen or stressful situations were intensified by sleep loss (Zohar, Tzischinsky, Epstein & Lavie,
2005). It is also possible that changes occur in the neuroendocrine stress systems under chronic sleep restriction (Meerlo, Sgoifo & Suchecki, 2008).

Many psychological factors can influence sleep. For example, research has found that people who perceive themselves as more socially isolated report more sleep problems (Steptoe, Owen, Kunz-Ebrecht & Brydon, 2004), and sleep laboratory research has shown they have worse sleep quality (take longer to get to sleep, and have more disturbances) than people who perceived themselves to be more socially connected (Cacioppo & Hawkley, 2003). Depression has also been linked to sleep disturbances, and one of the criteria for a depressive episode included alterations to sleep patterns (Franzen & Buysse, 2008). Lack of sleep is also thought to be a risk factor as well as a maintaining factor of depression (Kaneita et al., 2006; Riemann & Voderholzer, 2003). Anxiety, like depression has also been linked with poor sleep patterns (Uhde, Cortese & Vedenipin, 2009). Other negative psychosocial factors similarly, have an effect on sleep. Research indicates that forgiveness after negative interpersonal interactions is related to better sleep quality; whereas maintaining negative feelings, hostility and anger is related to poor sleep quality (Stoia-Caravello et al., 2008).

Similar research has found that high levels of anger are associated with sleep disturbances (Shin et al., 2005).

Sleep is a particular concern for people during the post-operative period. Sleep disturbances are commonly reported in hospital settings (Aurell & Elmquist, 1985; Friese, Diaz-Arrastia, McBride, Frankel & Gentilello, 2007; Simpson, Lee & Cameron, 1996) and may be complicated by stress stemming from the hospital environment and illness (Knapp-Spooner & Yarcheski, 1992).

Furthermore, pain also impacts sleep and this is also a common problem for post-operative patients (Simpson et al., 1996). Pain and sleep have a bi-directional association, with research showing that pain disturbs sleep but also that disturbances in sleep contribute to elevated experiences of pain (Edwards, Almeida, Klick, Haythrontwaite & Smith, 2008; Moldofsky, 2001). Sleep deprivation has been found to produce hyperalgesic changes and interferes with analgesic treatment (Lautenbacher, Kundermann & Krieg, 2006).
It is not surprising that sleep has an impact on wound healing and should be considered when assessing the links between psychological stress and healing. Research has found that animals that undergo sleep deprivation have delayed wound healing (Evans & French, 1995; Gümüstekin et al., 2004). In humans it has been found that sleep deprivation negatively affects skin barrier recovery and results in alterations in pro-inflammatory cytokines and natural killer cell activities (Altemus et al., 2001). Other research looking at wound healing has found that in the week before wounding, participants that reported more sleep had significantly faster healing time (Koschwanez et al., 2013). This research illustrates that not only do psychosocial factors have an influence on wound healing but they are also intertwined with other indirect factors such as sleep that may mediate healing. Lack of sleep can be an influence in its own right on wound healing or can exacerbate poor wound healing when under stress.

**Indirect effects of stress and health behaviours**

Negative health behaviours can result from stress and may mediate the effects of stress on healing. For example, increased alcohol consumption, poor nutritional intake, lack of exercise and smoking can all be linked with stress and other negative psychological states (Baum & Poslusny, 1999; Vitaliano et al., 2002). Research has found that depressed patients are more likely to smoke and less likely to quit compared to non-depressed patients (Wulsin, Vaillant & Wells, 1999).

In terms of wound healing, research has found that post-operative patients who smoke have slower wound healing and increase their risk of complications (Ahn & Mulligan, 2008; Chan, Withey & Butler, 2006; Sørensen, Hørby, Friis, Pilsgaard & Jørgensen, 2002). In animal models, administration of nicotine has been found to delay wound healing (Gümüstekin et al., 2004). This is because nicotine stimulates the sympathetic nervous system, resulting in the release of catecholamines, which causes vasoconstriction and decreases tissue blood perfusion. In addition, carbon monoxide in cigarette smoke causes tissue hypoxia, preventing oxygen from reaching tissue (Guo & DiPietro, 2010).
Similarly, alcohol consumption is linked to poor wound healing and risk of infection (Gentilello et al., 1993; Szabo & Mandrekar, 2009). Alcohol consumption results in suppression of pro-inflammatory cytokines, decreased neutrophil activity, delays in cell migration and deposition of collagen (Benveniste & Thut, 1981; Greiffenstein and Molina, 2008; Nelson, Bagby, Bainton & Summer, 1989). It is also common for people who smoke and engage in heavy alcohol consumption to have nutritional defects. Nutrition plays a role in wound healing, as glucose, polyunsaturated fatty acids, protein, and the vitamins A, C, E, and Zinc are essential dietary components during the healing progress (Russell, 2001; Scholl & Langkamp-Henken, 2001). Often people who are stressed make poor dietary decisions (Oliver & Wardle, 1999) and this is another indirect effect of stress that may also compromise wound healing.

Finally, lack of physical exercise may have a negative impact on wound healing. This is supported by a number of studies conducted with mice showing that increased physical exercise decreased inflammation and improved time to heal in comparison to control mice that did not exercise (Keylock et al., 2008; Pence, DiPietro & Woods, 2012). Similarly, research with older adults has found that those randomised to an exercise activity group had significantly faster healed wounds compared to a control group (Emery, Kiecolt-Glaser, Glaser, Malarkey & Frid, 2005).

Summary and conclusions

Overall these findings indicate that psychological stress impairs immunity via activation of the stress response, causing a significant delay in the healing process. The studies that have been conducted in this field repeatedly show the negative effects of stress on healing in difference scenarios using different measures of healing. Collectively, this research implicates the importance of addressing stress levels in patients that are undergoing clinical procedures or have chronic wounds in order to promote healing.

The pathways via which stress can influence healing are complex. At present not all of the mechanisms that underpin the link between stress, psychology and wound healing are understood.
Stress influences not only physiological but also behavioural responses such as sleep, alcohol use and exercise. Furthermore, other psychosocial factors such as depression, anger and social isolation can also alter the neuroendocrine pathways associated with the stress response, as well as physiological and behavioural responses. This illustrates the number of ways in which psychosocial factors can alter healing.

Given the robust evidence that psychological factors influence healing, research should head towards investigating ways to alter psychological factors to positively influence wound healing. By conducting research in healthy populations with standardised wounds, interventions can be developed and tested before applying such findings to clinical populations. To date some interventions have already been trialled and the next chapter presents a systematic review of psychological interventions and wound healing.
Chapter 3: The effects of psychological interventions on wound healing: A systematic review of randomised trials

Preface

Rationale for experimental research: Systematic review

As described in Chapter 2, the direct effects of stress on the neuroendocrine pathways can influence wound healing. In addition, other negative psychological factors, such as anger, depression, and loneliness may also activate these pathways causing impaired wound healing. Finally, wound healing can be affected by physiological process such as pain and sleep that serve to exacerbate or delay wound healing both directly and indirectly by amplifying stress or stress related behaviours. As of yet there is very little research that has looked at whether psychological interventions to improve psychological states can improve wound healing.

Research indicates that psychological interventions to improve physical health outcomes help by improving mood and facilitating adaptive coping strategies for distressful thoughts and events. These are broadly categorized below as stress reduction interventions, cognitive restructuring interventions and interventions to alter perceptions or expectations.

Stress reduction techniques include relaxation exercises, which includes deep breathing exercises, muscle relaxation, guided imagery, mindfulness meditation (mediation focusing on abdominal breathing and accepting in a non-judgemental way distracting cognitions), and gentle stretching exercises, such as yoga or tai chi, that focus on breathing and reducing muscle tension. Experimental research indicates that relaxation interventions can decrease salivary cortisol levels and perceived stress (Kamei et al., 2000; Lucini et al., 1997; Pawlow & Jones, 2005; Smith, Hancock, Blake-Mortimer & Eckert, 2007; Zachariae et al., 1990). As well as decreasing cortisol levels, relaxation interventions have been shown to reduce pain and improve recovery after surgery (Good, Anderson, Ahn, Cong & Stanton-Hicks, 2005; Roykulcharoen & Good, 2004), and improve sleep
latency and sleep duration (Kim & Kim, 2005; Wright, Wright, Courtney & Crowther, 2002), both factors known to influence wound healing.

Psychological interventions that focus on cognitive restructuring through thought identification and replacement have been found to have both immunological and psychological effects. For example, cognitive behavioural stress management programmes that aim to not only teach relaxation skills but also target thought patterns have been found to reduce cortisol levels, anxiety and mood disturbances (Cruess, Antoni, Kumar & Schneiderman, 2000; Phillips et al., 2008). Similarly expressive writing helps process upsetting thoughts around a stressful event, resulting in decreased distress, improved mood and decreased rumination (Pennebaker, 1997). This is because suppression of emotional thoughts has been shown to increase autonomic system arousal (Gross & Levenson, 1993) and keeping emotions contained over an extended period of time can compromise immune competence and lead to poor physical health (Pennebaker, 1989; Temoshok, 1986).

Expressive writing has been linked to improvements in immune functioning (Booth, Petrie & Pennebaker, 1997; Esterling, Antoni, Fletcher, Marguiles & Schneiderman, 1994) and decreases in cortisol (Smyth, Hockemeyer & Tulloch, 2008). Furthermore, like other psychological interventions expressive writing has been linked to decreased pain and better mood (Francis & Pennebaker, 1992; Greenberg & Stone, 1992), as well as improved sleep (Arigo & Smyth, 2012; Gillis, Lumley, Mosley-Williams, Leisen & Roehrs, 2006).

Finally psychological techniques to alter perceptions or expectations about the procedure and/or recovery, particularly for patients undergoing a surgical or a medical procedure can be useful. Distraction techniques help to minimize perceptions of fear and discomfort leading to less anxiety during the procedure and recovery and less pain. Distraction techniques such as music, audio visual devices, toys and video games are often employed in paediatric settings (Arts et al., 1994; Vessey, Carlson & McGill, 1994) but are also useful in other setting. For example, audio visual devices are often used during dental procedures to reduce anxiety and pain that shortens the procedure time.
(Frere, Crout, Yorty & McNeil, 2001). Similarly, in burns patients, watching a distracting video during dressing changes reduces pain and anxiety (Miller, Hickman & Lemasters, 1992).

Similar to distraction interventions, placebo effects and hypnosis strategies both aim to alter expectations about pain and anxiety by using suggestion. Research has found that placebos can diminish pain perception (Colloca & Benedetti, 2005; Montgomery & Kirsch, 1996), illustrating how the brain plays a role in providing external, top-down control that modulates physiological outcomes. Similarly patients under hypnosis report significantly less pain during medical procedures and recovery (Patterson, Everett, Burns & Marvin, 1992; Syrjala, Cummings & Donaldson, 1992). Research has found that hypnosis and placebo can alter neuroendocrine reactivity (Colloca & Benedetti, 2007; Wood et al., 2003), implying that changes in perceptions and expectation promote psychological well-being that is translated into reduced physiological reactivity leading to improvements in immunological functioning (Gruzelier, Smith, Nagy & Henderson, 1997; Neumann, 2005; Ruzyla-Smith, Barabasz, Barabas & Warner, 1995).

At present there has been no review conducted collating the literature to date that has explored the potential benefits of psychological interventions on wound healing specifically. However, surgical recovery has been extensively researched. A meta-analysis conducted by Devine (1992) included 191 studies that used psychosocial interventions with surgical patients on other surgical outcomes. These interventions could be categorised as healthcare education (details about what to expect before and after surgery and explanation of surgical procedures), teaching of skills (such as relaxation, exercises and breathing) or psychosocial support (providing reassurance, listening to patient concerns). Specifically, almost 80% of the studies indicated a positive benefit as a result of intervention and length of stay was decreased on average by 1.5 days. Similarly, Johnston and Vögele (1993) conducted a meta-analysis with 38 studies, which examined the effects of procedural information, relaxation, hypnosis and emotion focused approaches on surgical outcomes. The results found that the strongest evidence existed for procedural information and relaxation.
interventions, with benefits in various outcomes including pain, length of stay, mood and clinical indicators of recovery. Although some of these interventions are quite different, making it hard to determine mechanisms behind their effect, they show that psychological and physical outcomes are linked. Another recent review conducted by Nelson et al. (2013) focused primarily on interventions that have been found to be useful for surgical patients including relaxation, mindfulness, hypnosis and guided imagery. They found that of the 20 studies investigated there was strong evidence for improvements in psychological well-being as a result of relaxation interventions, while only partial evidence supporting hypnosis. A common limitation noted in previous literature reviews has been the quality of studies included in each review and this should be taken into account in the conclusions.

As well as examining psychosocial interventions that predict better surgical outcomes, it is relevant to review research that has looked at the influence of such factors on immune functioning in order to inform what types of interventions would have beneficial effects on wound healing. A systematic review conducted by Miller and Cohen (2001) looked at the efficacy of psychological interventions in immune modulation. They included 85 studies in their review and reported that there is some evidence for immune modulation as a result of psychological interventions. They reported that the strongest evidence comes from hypnosis research and trials that used conditioning as a technique. They described that stress management interventions such as deep breathing showed mixed results. However, despite the limited evidence to alter immune responses the authors state that in light of the methodological issues of various studies, the results should not be interpreted in a way that suggests that psychological interventions are of limited use. More recently Wahbeh and colleagues (2009) conducted a systematic review of psychological interventions that assessed immunological outcomes. One-hundred and one studies were included in the review that included healthy patients as well as patients with HIV, cancer and allergies. Most studies looked at either relaxation training, cognitive stress management or hypnosis. They found that relaxation interventions showed the best outcomes for immune function.
Taken together the literature discussed suggests that psychological interventions have potential to improve wound healing. It is important to assess wound healing as an outcome because wound healing can be objectively measured and translated into health outcomes and recovery. By assessing wound healing, the influence of psychological factors on physiological processes can be investigated. The mechanisms via which such interventions operate include reductions in the stress response as well as reductions in the interpretation of distressing events as overwhelming. In reducing physiological symptoms of stress as well giving people strategies to deal with or reframe stressful situations, wound healing may be enhanced. However, as of yet, a systematic review of studies investigating the effects of psychological interventions on wound healing has not been conducted.

**Aims**

The aims of the systematic review described here in Chapter 3 were:

1. To collate and assess the research conducted looking at the effects of psychological interventions on health, measuring wound healing as outcome.
2. To determine the gaps in the current literature and identify the strengths and weaknesses in the studies conducted to date in order to guide future research.

**Citation**

Abstract

Background: Psychological stress has been shown to delay wound healing. Several trials have investigated whether psychological interventions can improve wound healing but to date this evidence base has not been systematically synthesised. The objective was to conduct a systematic review of randomized controlled trials in humans investigating whether psychological interventions can enhance wound healing.

Methods: A systematic review was performed using PsychINFO, CINAHL, Web of Science and MEDLINE. The searches included all papers published in English up until September 2016.

The reference lists of relevant papers were screened manually to identify further review articles or relevant studies. Nineteen studies met inclusion criteria and were included in the review.

Results: Fifteen out of nineteen studies were of high methodological quality. Six studies were conducted with acute experimentally created wounds, five studies with surgical patients, two studies with burn wounds, two studies with fracture wounds, and four studies were conducted with ulcer wounds. Post-intervention standardised mean differences (SMD) between groups across all intervention types ranged from 0.13 to 3.21, favouring improved healing, particularly for surgical patients and for relaxation interventions. However, there was some evidence for publication bias suggesting negative studies may not have been reported. Due to the heterogeneity of wound types, population types and intervention types it is difficult to pool effect sizes across studies.

Conclusions: Current evidence suggests that psychological interventions may aid wound healing. Although promising, more research is needed in order to assess the efficacy of each intervention on different wound types.
Introduction

Research over the past twenty years has consistently demonstrated that psychological stress impairs wound healing (Christian, Graham, Padgett, Glaser & Kiecolt-Glaser, 2006; Walburn, et al., 2009). Effects have been shown for both acute and chronic wounds (Ebrecht et al., 2004; Marucha, et al., 1998; Vedhara et al., 2010).

There are several pathways by which stress may impact healing. First, it may have direct effects via the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal-medullary axes (SAM; Godbout & Glaser, 2006). The HPA and SAM axes regulate the release of pituitary and adrenal hormones including cortisol and catecholamines (epinephrine and norepinephrine). The release of cortisol and catecholamines can cause changes in cellular trafficking, proliferation, antibody production and cytokine secretion (Padgett & Glaser, 2003). Chronic stress diminishes pro-inflammatory cytokines, matrix metalloproteinases and neutrophil infiltration at the wound site, all of which are critical in the early phases of wound repair (Gouin & Kiecolt-Glaser, 2011). Other negative mood states have been linked to similar neuroendocrine changes as result of the activation of the HPA and SAM axes (Miller, 1998). For example, depression and anxiety can alter immune function by affecting the production of pro-inflammatory cytokines (Dentino et al., 1999; Lutgendorf et al. 1999).

Another route by which stress, anxiety and depression may affect wound healing is indirectly via health behavioural patterns (poor dietary choices, smoking, alcohol, lack of sleep and lack of exercise, poor adherence to medications). Furthermore, these mood states amplify pain, which also affects the release of stress-related hormones (Kiecolt-Glaser et al., 2002). Pain is particularly relevant in the context of healing because injury will be associated with some level of distress. High levels of pain or expected pain not only elevate distress levels, but also interfere with the healing process (Widgerow & Kalaria, 2012).

Given the deleterious effects of stress and negative emotions on wound healing, research has begun to investigate whether or not psychological interventions can improve wound healing.
Psychological interventions may reduce negative mood states and therefore reduce alterations in HPA and SAM activity which may translate to faster wound healing. Interventions may also promote adaptive behavioural coping strategies, such as quitting smoking.

Psychological interventions have been found to reduce stress and anxiety, physiological arousal, HPA activity, catecholamine levels and alter expectations about pain and recovery in healthy populations, cancer patients and surgical patients (Antoni et al., 2000; Biondi, & Picardi, 1999; Brunges & Avigne, 2003; Carlson, Speca, Faris & Patel, 2007; Cruess et al., 2000; Curtis, Osadchuk & Katz, 2011). A meta-analysis by Miller and Cohen (2001) concluded that under the appropriate circumstances, psychological interventions show promise to reliably improve immune function in various illnesses. The review suggested that most evidence comes from hypnosis interventions, with some positive findings for emotional disclosure, stress management and relaxation. More recently, Wahbeh and colleagues (2009) found that relaxation interventions showed the best outcomes for immune function. Because the immune system is integral to wound healing, such interventions may also be able to improve wound healing, and have important implications for clinical populations such as surgical patients. Reviews have shown that psychological interventions can alleviate pain and anxiety after surgery but have not included wound healing as an outcome (Good, 1996; Kekecs, Nagy & Varga, 2013; Nelson et al., 2013). To date, several trials have investigated the effects of psychological interventions on wound healing, however no systematic reviews have been conducted in this area.

The aim of this review was to systemically identify and collate randomized controlled trials investigating the effects of psychological interventions on wound healing in humans. The results of this process will show which types of interventions have been tested, whether the studies have been methodologically sound, which interventions show the best preliminary evidence for clinical implementation, and where more research is needed.
Methods

A systematic review was conducted of psychological interventions that measured wound healing as an outcome. The review is reported using the PRISMA guidelines (http://www.prisma-statement.org/) (Moher, Liberati, Altman & Tetzlaff, 2009).

Eligibility

Studies were included if they met the following criteria: 1) randomized control trials (RCTs) manipulating psychosocial factors; 2) inclusion of a control group; 3) measured healing as an outcome; and 4) written in English. This review used the definition of a wound as a “disruption of normal tissue structure and function” (Walburn et al., 2009). A variety of wound types were included in this review, including experimentally created wounds or clinical wounds, both chronic and acute. Studies assessing the permeability of the epidermis as a result of tape stripping were also included because tape stripping creates an imbalance in the tissue structure and function. Both healthy and clinical populations were included. This review included all published work up until 30 September 2016. Studies reported as abstracts in conferences were not included.

Studies that were conducted with animals were not included in this review because psychological factors explored in animal models, such as social housing or environment enrichment are qualitatively different to methods used in studies using human participants. Studies that aimed to improve well-being by directly modulating anatomical and/or physiological processes (e.g. exercise therapy, medication) were excluded similar to Miller and Cohen (2001). Research that focused on spiritual healing practices, such as prayer, non-contact therapeutic touch and distant healing were also excluded. We chose to exclude these studies because clearly manualised instructions of such interventions are rarely reported (similar to Nelson et al., 2013).

Search strategy

A literature search was performed using PsychINFO, CINAHL, Web of Science and MEDLINE. The searches included all papers published in English up until 30th September 2016. The primary
search was conducted with the following pattern: (“Wound healing” OR “Skin barrier recovery” OR “Transepidermal water loss” OR “Reepithelialisation”) AND (“Psychological intervention” OR “Psychological treatment” OR “Relaxation” OR “Guided imagery” OR “Mindfulness” OR “Hypnosis” OR “Distraction” OR “Yoga” OR “Emotional disclosure” OR “Expressive writing” OR “Social support” OR “Placebo” OR “Expectancy”). Psychosocial search terms were based on key words used in previous reviews and known psychosocial variables associated with immunity, recovery and healing (Miller & Cohen, 2001; Nelson et al., 2013). Additionally, the reference lists of relevant papers were screened manually to identify further review articles or relevant studies.

Study selection

After the search had been performed, and duplicates removed, titles and abstracts were screened to identify relevant articles in accordance with the inclusion criteria. The full manuscripts of all studies deemed to be relevant were obtained. Studies that met the inclusion criteria were further reviewed by two researchers to qualify them for inclusion in the analysis.

Data were extracted using a comprehensive, pre-designed spreadsheet. Extracted information included: (1) participant characteristics (total number, age, gender); (2) type of wound sustained (ulcer, surgery, biopsy, burn, fracture, blister, tape stripping, laser ablation); (3) type of wound assessment method used; (4) type of intervention or psychosocial factor investigated (type, description, frequency); (5) control group details; (6) main data collection-points and (7) statistical significance of findings and effect size.

Effect size

The extracted data was converted to standardized mean differences (SMD). To aid comparison across continuous and categorical outcomes, data from categorical outcomes was also converted to an SMD metric using the logit method (Lipsey & Wilson, 2001). Caution should be taken with such comparisons because outcomes measured on different scales are not directly comparable even when expressed using the same metric. The SMD expresses the size of the intervention effect.
in each study relative to the variability observed in that study, as indicated by the pooled standard deviation (Lispey & Wilson, 2001). SMD and standard error was calculated using an online calculator. (http://www.campbellcollaboration.org/resources/research/effect_size_calculator.php)

Due to the level of design heterogeneity in terms of the type of wound sustained, type of wound assessment, and type of psychological intervention it was not deemed appropriate to calculate an overall pooled estimate of the effect of psychological interventions on wound healing. Furthermore, due to the small number of studies and design heterogeneity with respect to the type of wound assessed it was also not possible to calculate pooled effect sizes within any intervention type subgroupings.

**Quality assessment and publication bias**

To assess the quality of the eligible studies two reviewers working independently analysed each study using the 27-point Downs and Black Quality Index (Downs & Black, 1998). The quality index highlights the vulnerabilities in design, with regard to reporting external validity, bias, and confounding factors. The checklist focused on four areas: Quality of reporting (i.e. aims, methods and findings have been clearly and accurately described); external validity (i.e. assessing the representativeness of the study findings and generalizability to larger population); internal validity bias (i.e. use of blinding and appropriate statistical tests used); and internal validity selection bias (i.e. randomized recruitment of participants). Reviewers reached 100% agreement for the quality assessment for each paper and the quality assessment is expressed as a percentage. Publication bias was assessed by visually evaluating a funnel plot of the trial mean differences in asymmetry of all studies included. Due to heterogeneity, the pooled SMD and its pseudo 95% confidence intervals calculated are not appropriate reflections of these properties and should be ignored. Rather the intention is to identify asymmetry in the effect sizes reported – specifically whether small negative findings appear to be missing from the literature. Egger’s test for bias was not conducted, because the studies included in the analysis were not pooled.
Results

Study selection

The combined search yielded a total of 4,490 articles. After duplicates were removed 3,670 articles remained. Further screening of the remaining articles identified 96 potential articles and full-text articles were retrieved. After review, a total of 19 articles were included in the analysis. Table 1 provides a summary of the 19 studies included in this review.

Study characteristics

A total of 1,114 participants took part in the 19 studies (620 females, 56%). Individual sample sizes ranged from 12 to 121 participants (Mean = 58.63, SD = 33.64). The average age amongst the 14 studies that reported the average (not just the range), varied between from 6.01 years to 78.8 years. The overall average age across these 14 studies was 37.15 (SD = 20.33) years.

Quality assessment of studies

As seen in Table 1, the quality of assessment for each study was relatively high, with an average quality rating of 73.7% (SD = 9.3) ranging from 48.2% to 81.5%. Fifteen of the 19 studies rated higher than 70%.
<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Participants</th>
<th>Intervention</th>
<th>Type of surgery/wound</th>
<th>Main wound healing outcome</th>
<th>Data collection points</th>
<th>Outcomes</th>
<th>Study quality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimentally created wounds</strong></td>
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<tr>
<td>Gouin, Kiecolt-­Glaser, Malarkey &amp; Glaser, 2008</td>
<td>N = 98 (58 females)</td>
<td>During the experimental session received either: a) 45 minute relaxation session (deep breathing, progressive muscle relaxation, imagery and self-hypnosis) after blister procedure had begun, then a session 1, 4 and 8 hours later. b) no relaxation (quiet reading)</td>
<td>Blisters created on arm using a suction blister device to separate the dermal-epidermal junction, creating eight 8 mm blisters.</td>
<td>Day to heal based on 90% return to TEWL baseline criterion</td>
<td>Daily for 8 days</td>
<td>No significant differences in healing between the relaxation group and the control group (p = .53)</td>
<td>62.96</td>
</tr>
<tr>
<td>Koschwanez, Kerse, Darragh, Jarrett, Booth &amp; Broadbent, 2013</td>
<td>N = 49 (28 females) 78.8 (7.2) years</td>
<td>Two weeks prior to biopsy completed either: a) expressive writing task (done over 3 days, 20 minutes a day) about thoughts/emotions around an upsetting event b) time management writing task (done over 3 days, 20 minutes a day) about how they spent their time</td>
<td>4mm punch biopsy of inner arm</td>
<td>% of participants who had full re-epithelialisation on each day. Photographs rated as re-epithelialisation or not by dermatologist</td>
<td>Days 7, 11, 14,17 and 21 post-biopsy</td>
<td>Significantly more participants in the expressive writing group had full re-epithelialisation on day 11 than time management group (p = .028)</td>
<td>77.78</td>
</tr>
<tr>
<td>Robinson, Jarrett &amp; Broadbent, 2015</td>
<td>N = 121 (87 females) 23.9 (9.9) years</td>
<td>During experimental session received either: a) 20 minute relaxation session (deep breathing, progressive muscle relaxation prior to tape stripping b) 20 minute relaxation session (deep breathing, progressive muscle relaxation after to tape stripping c) no relaxation (quiet reading)</td>
<td>Tape stripping of the inner forearm</td>
<td>% skin barrier recovery represented by TEWL</td>
<td>25 minutes after tape stripping was completed</td>
<td>Significantly faster healing in both relaxation groups compared to no relaxation (p = .032)</td>
<td>81.48</td>
</tr>
<tr>
<td>Robles, 2007</td>
<td>N= 85 (44 females) 22.9 (4.4) years</td>
<td>After tape stripping procedure completed either: a) stress task with no support b) stress task with support from confederate during preparation period c) no stress (read magazine article)</td>
<td>Tape stripping of the inner forearm</td>
<td>% skin barrier recovery represented by TEWL</td>
<td>60, 90 and 120 minutes after tape stripping was completed</td>
<td>Significantly faster healing in the no stress group compared to both groups that were stressed (p = .007). The addition of social support did not significantly influence healing when compared to those who underwent the stress task without social support (p = .30)</td>
<td>77.78</td>
</tr>
<tr>
<td>Vits, Dissemond, Schadendorf, Kriegler, Korber, Schiedlowski &amp; Cesko, 2013</td>
<td>N = 23 (all male) 29.6 (5.9) years</td>
<td>Post-procedure received either: a) placebo gel (participants told it would accelerate wound healing and reduce pain) on one thigh and non-active gel on the other thigh (both gels actually the same) b) same on-active gel applied to both</td>
<td>1cm squared, 2mm deep wounds created by ablative laser on both thighs</td>
<td>Wound healing assessed by planimetry, where area of wound non re-epithelialised was calculated</td>
<td>Day 1, 4, 7 and from Day 9 onwards daily</td>
<td>No significant differences in healing between wounds treated with placebo gel or non-active gel observed</td>
<td>59.26</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>Age (SD)</td>
<td>Intervention</td>
<td>Outcome</td>
<td>Healing Difference</td>
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</table>
| Weinman, Ebrecht, Scott, Walburn & Dyson 2008 | 36    | 22.2 (4.1) years | Two weeks prior to biopsy completed either:  
  a) expressive writing task (done over 3 days, 20 minutes a day) about thoughts/emotions around an upsetting event  
  b) time management writing task (done over 3 days, 20 minutes a day) about how they spent their time | 4mm punch biopsy of inner arm  
  Wounds scanned to measure width at base (mm) | Days 7, 14 and 21 | Significantly faster healing in expressive writing group compared to time management group (p < .05) |
| Surgical wounds                      | 60    | 51.3 (16.75) years | At least 3 days prior to surgery and 7 days post-surgery received either:  
  a) 45 minute relaxation session before surgery and 20 minute audiotape to listen to prior to surgery and after surgery that included deep breathing, progressive muscle relaxation and guided imagery  
  b) usual care | Elective laparoscopic cholecystectomy  
  Collagen deposition as measured by hydroxyproline | Baseline (prior to surgery) and 1 week post-surgery | Significantly higher amounts of collagen deposition in the relaxation group compared to usual care indicating faster healing (p = .02). |
| Ginandes, Brooks, Sando, Jones & Aker, 2003 | 18    | 38.9 (8.7) years | Starting 2 weeks prior to surgery and continuing 6 weeks post-surgery received either:  
  a) Eight 30 minute adjunctive supportive sessions (thoughts about surgery, impact on life and available support)  
  b) Eight 30 minute adjunctive hypnosis sessions (suggestions to reduce inflammation, pain, stimulate healing)  
  c) usual care (no additional sessions) | Reduction mammaplasty surgery  
  Nurses rating of WAI - 3 subscales (edema, erythema and open wound site). Rated out of 3 | Week 7 post-surgery | Significantly faster healing in adjunctive hypnosis group compared to usual care and adjunctive support group (p < .001). Not reported statistical significance between usual care and adjunctive support group although data indicates that the adjunctive support group showed faster healing than usual care. |
| Holden-Lund, 1988                     | 24    | 47.5 (13.75) years | One day prior to surgery and 3 days post-surgery received either:  
  a) 20 minute relaxation audiotape to listen to prior to surgery and 3 days after surgery that included deep breathing, progressive muscle relaxation and guided imagery  
  b) 20 minute quiet period prior to surgery and 3 days after surgery | Elective cholecystectomy  
  Nurse and surgeon ratings of WAI - 3 subscales (edema, erythema and exudate). Rated out of 3 | Day 3 | Significantly lower ratings of erythema in the relaxation group than the control group indicating faster healing (p < .010) |
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Sample Size</th>
<th>Description</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pereria, Figueiredo-Braga &amp; Carvalho, 2016</td>
<td>2016</td>
<td>N = 104 (54 females) 43.7 (14.5) years</td>
<td>At pre-surgical nursing interview received either: a) standardized information about hospitalization and surgical preparation procedures b) empathetic patient centred interviewing approach where nurses explored patients' personal concerns about the surgery, leading to a sense of validation and understanding</td>
<td>Inguinal Hernia Repair, Hemorreideectomy, Pilonidal sinus resection.</td>
<td>Type of wound tissue subscale of Pressure Ulcer Scale for Healing (PUSH). Rated out of 4 by nurses</td>
<td>1 month post-surgery</td>
</tr>
<tr>
<td>Rao, Nagendra, Raghuram, Vinay, Chandrashekar, Gopinath &amp; Srinath, 2008</td>
<td>2008</td>
<td>N = 98 (all females) 49.2 (9.6) years</td>
<td>Prior to surgery and over 4 weeks post-surgery received either a) 60 minute introductory session prior to surgery and 4 bedside supportive counselling sessions (education, social support and rehabilitative shoulder exercises) to be continued for 3 weeks after discharge. b) 60 minute introductory session prior to surgery and 4 bedside yoga sessions (deep breathing and yogic relaxation techniques) to be continued daily for 3 weeks after discharge</td>
<td>Breast cancer surgery (masectomy or breast conservation)</td>
<td>Number of days from surgery to suture removal (removed when primary union was facilitated, indicates that wound margins are closed)</td>
<td>Baseline (prior to surgery) and 4 weeks post-surgery</td>
</tr>
<tr>
<td>Burns Brown, Kimble, Rodger, Ware &amp; Cuttle, 2014</td>
<td>2014</td>
<td>N= 75 (39 females) 8.3 (2.6) years</td>
<td>During burn dressing received either: a) multi-modal distraction (handheld device with both protocol of procedural preparation and distraction in the form of touch games and stories) b) standard distraction (television, stories, toys)</td>
<td>An acute burn of any depth less than 15% of the body (excluding superficial erythema burns)</td>
<td>The number of days from the date of the burn until 95% reepithelialisation was measured by Visitrak (wound tracing device)</td>
<td>Daily dressing changes until healed</td>
</tr>
<tr>
<td>Miller, Rodger, Kipping &amp; Kimble, 2011</td>
<td>2011</td>
<td>N = 40 (19 females) 6.01 (2.24) years</td>
<td>During burn dressing received either: a) multi-modal distraction (handheld device with both protocol of procedural preparation and distraction in the form of touch games and stories) b) standard distraction (television, stories, toys)</td>
<td>An acute burn of any depth less than 15% of the body (excluding superficial erythema burns)</td>
<td>Days to heal assessed by doctor. Burn classed as healed when it was re-epithelialised and only required moisturiser at the dressing change</td>
<td>Daily dressing changes until healed</td>
</tr>
<tr>
<td>Fracture Ginandes &amp; Rosenthal, 1999</td>
<td>1999</td>
<td>N = 12 (9 females)</td>
<td>Post-immediate orthopedic care over 12 weeks received either: a) 6 standard care appointments b) 6 standard care appointments plus 6 hypnosis sessions with psychologist (suggestions to reduce inflammation, pain, stimulate healing) and audio tape to take home</td>
<td>Malleolar (ankle) fracture</td>
<td>Fracture edge (graded compared to normative healing and assigned a number based on week that healing appeared to be in).</td>
<td>Week 3, 6, 9 and 12 following fracture</td>
</tr>
<tr>
<td>Study</td>
<td>Sample Size</td>
<td>Intervention</td>
<td>Outcome Measure</td>
<td>Findings</td>
<td></td>
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<tr>
<td>Oswal, Raghuram, Ebnezar &amp; Nagendra, 2011</td>
<td>N = 30 (8 females) 34.7 (13.9) years</td>
<td>Post-immediate orthopedic care over 12 weeks received either: a) standard care b) Seven 30 minute yoga training sessions for 1 week (deep breathing and directing energy prone to the fracture site) and then practiced yoga twice a day for 3 weeks using audiotapes</td>
<td>Simple extra-articular fractures of long and short bones Fracture line density (rating 1-4) Number of broken cortices (rating 1-4) Week 1 and week 3</td>
<td>Significantly faster healing in yoga group compared to standard care for both fracture line density (p &lt;.001) and number of broken cortices (p = .001). 77.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach ulcer Han, 2002</td>
<td>N= 47 (20 females)</td>
<td>Over 4 months received either: a) 15 minute progressive muscle relaxation audio tape to listen to at home b) integrated stress management program consisting of seven 1 hour sessions over a 4-week period (biofeedback, progressive muscle relaxation and cognitive emotional coping strategies)</td>
<td>Participants diagnosed with a peptic stomach ulcer Ulcer healing rated by physician using gastroendoscope giving a score from 1 to 6 (1=white scar, 2=red scar, 3=healing process 2, 4=healing process 1, 5=active regression stage, or 6=active acute stage) Baseline and 4 months later</td>
<td>Significantly faster healing in the integrated stress management group compared to the progressive muscle relaxation (p = .05) 62.96</td>
<td></td>
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</tr>
<tr>
<td>Wu, Guo, Gao, Kang, Guo, Jiang, Chen, Liu &amp; Li, 2012</td>
<td>N= 96 (21 females) 20 to 59 years</td>
<td>Over 6 weeks received either: a) Cognitive therapy (change illness perceptions towards ulcer and curability) b) standard care</td>
<td>Participants diagnosed with a peptic stomach ulcer Gastroscopy performed so that ulcers could be rated as 1-recovered (no ulcer lesion left), 2-effective (ulcer shrunk more than 50%) or 3 - ineffective (ulcer shrunk less than 50%). Results reported as % healed Baseline and 6 weeks later</td>
<td>Significantly faster healing in cognitive therapy group compared to standard care (p &lt;.05) 48.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic ulcer Edwards, Courtney, Finlayson &amp; Shuter, 2009</td>
<td>N= 66 (31 females)</td>
<td>Over 6 months received either: a) individual home visits b) weekly visits to Lindsay Leg Club (peer support)</td>
<td>Venous leg ulcers % reduction in area from baseline ulcer area reduction from baseline Baseline, 12 weeks and 24 weeks</td>
<td>Trend for Lindsay Leg club group to show larger reduction in percentage reduction of ulcer area over 24 weeks but this is not significant (p = .135). Significantly reduction in ulcer area in Lindsay Leg club group over 24 weeks (p = .004) 74.04</td>
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</tbody>
</table>
| Rice, Kalker, Schindler & Dixon, 2001 | N= 32 (19 females) 63.1 (13.6) years | Over 3 months received either: a) one training session in biofeedback assisted relaxation (progressive muscle relaxation, deep breathing, autogenic training) and a 16 minute audiotape to listen to 5 times a week b) standard care | Non-healing chronic foot ulcers Ulcer healing by photographs (measured area to calculate healing rates i.e. "the change in wound area (mm2) over time (days)."

Baseline and 3 months later | Significantly faster healing in relaxation group compared to standard care in changes in wound area (p <.002) 77.78 |
**Publication bias**

Figure 1 shows the funnel plot of the studies included in this review. Due to the variability in the nature of interventions and wounds assessed, the funnel plot is only a crude indication of publication bias and the vertical line indicating the pooled effect should be ignored. Inspection of the figure reveals some asymmetry with fewer studies to the lower left hand side of the graph, which may indicate missing small negative studies in the published literature. Furthermore, on the extreme right hand side of the graph is a clear outlier. Asymmetry in the funnel plot may also have occurred because many trials included had small sample sizes where inherent biases may have led to an inflated estimate of intervention effects.

![Funnel plot with pseudo 95% confidence limits](image)

*Figure 1. Funnel plot of studies included in systematic review.*
Study outcomes

In the next sections, we present the results primarily based on wound type, but also by the kind of intervention used. Moderating variables are examined, including participant age and gender, intervention intensity and duration, compliance, timing of the intervention, and the wound assessment method. Finally, possible mediating factors are examined.

Figure 2 shows the effect sizes of studies included in the analysis. The studies can be categorised into acute and chronic wound types. Six studies utilized healthy populations with acute experimentally created wounds: tape stripping (2 studies), punch biopsy (2 studies), blister (1 study), and ablative laser wound (1 study). Of the remaining studies, five were conducted with surgical patients; two were conducted with burn wounds; two with fracture wounds and four with ulcer wounds (2 of these studies were conducted with chronic leg ulcer wounds and two with acute stomach ulcers). All of these wounds can be considered acute apart from the chronic leg ulcer wounds.
<table>
<thead>
<tr>
<th>Study</th>
<th>Treat</th>
<th>Outcome</th>
<th>SMD (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Broadbent (2012)</td>
<td>Relaxation</td>
<td>Collagen deposition, 7 days</td>
<td>0.61 (0.07, 1.15)</td>
</tr>
<tr>
<td>Holden-Lund (1988)</td>
<td>Relaxation</td>
<td>WAL, 3 days</td>
<td>0.71 (0.01, 1.42)</td>
</tr>
<tr>
<td>Gmandes (2003)</td>
<td>Support</td>
<td>WAL, 7 weeks</td>
<td>0.93 (-0.28, 2.13)</td>
</tr>
<tr>
<td>Gmandes (2003)</td>
<td>Hypnosis</td>
<td>WAL, 7 weeks</td>
<td>1.89 (0.49, 3.30)</td>
</tr>
<tr>
<td>Rao (2008)</td>
<td>Yoga</td>
<td>Days to suture removal</td>
<td>0.53 (0.05, 1.01)</td>
</tr>
<tr>
<td>Pereira (2016)</td>
<td>Empathetic care</td>
<td>PUSH, 1 month</td>
<td>0.56 (0.17, 0.95)</td>
</tr>
<tr>
<td>Tape stripping</td>
<td></td>
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</tr>
<tr>
<td>Robinson (2015)</td>
<td>Relaxation</td>
<td>% skin barrier recovery, 25 mins</td>
<td>0.47 (0.03, 0.92)</td>
</tr>
<tr>
<td>Robles (2007)</td>
<td>Support</td>
<td>% skin barrier recovery, trend</td>
<td>0.25 (-0.26, 0.76)</td>
</tr>
<tr>
<td>Ulcer</td>
<td></td>
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<tr>
<td>Rice (2001)</td>
<td>Relaxation</td>
<td>Change in area, 3 months</td>
<td>0.87 (0.14, 1.60)</td>
</tr>
<tr>
<td>Edwards (2009)</td>
<td>Support</td>
<td>% reduction in area, 24 weeks</td>
<td>0.36 (-0.12, 0.85)</td>
</tr>
<tr>
<td>Wu (2012)</td>
<td>CBT</td>
<td>% healed, 6 weeks*</td>
<td>0.55 (0.05, 1.05)</td>
</tr>
<tr>
<td>Han (2002)</td>
<td>Stress management</td>
<td>Ulcer healing, 4 months</td>
<td>0.57 (-0.01, 1.15)</td>
</tr>
<tr>
<td>Suction blister</td>
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</tr>
<tr>
<td>Gouin (2008)</td>
<td>Relaxation</td>
<td>Trans-epidermal water loss, 4 days</td>
<td>0.13 (-0.27, 0.53)</td>
</tr>
<tr>
<td>Burn</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown (2014)</td>
<td>Distraction</td>
<td>Days until 95% reepithelialisation</td>
<td>0.40 (-0.06, 0.86)</td>
</tr>
<tr>
<td>Miller (2011)</td>
<td>Distraction</td>
<td>Days to heal</td>
<td>0.67 (-0.56, 1.91)</td>
</tr>
<tr>
<td>Punch biopsy</td>
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<tr>
<td>Weinman (2008)</td>
<td>Expressive writing</td>
<td>Width of wound (mm), 2 weeks</td>
<td>0.77 (0.10, 1.45)</td>
</tr>
<tr>
<td>Koschwanez (2013)</td>
<td>Expressive writing</td>
<td>% reepithelialised, 11 days*</td>
<td>0.82 (0.07, 1.56)</td>
</tr>
<tr>
<td>Fracture</td>
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<tr>
<td>Gmandes (1999)</td>
<td>Hypnosis</td>
<td>Fracture line, 6 weeks</td>
<td>0.34 (-0.85, 1.54)</td>
</tr>
<tr>
<td>Oswal (2011)</td>
<td>Yoga</td>
<td>Fracture line, 3 weeks</td>
<td>3.21 (2.08, 4.33)</td>
</tr>
<tr>
<td>Laser ablation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vits (2013)</td>
<td>Placebo</td>
<td>Days to reepithelialisation</td>
<td>0.15 (-1.52, 1.82)</td>
</tr>
</tbody>
</table>

Figure 2: Forest plot of wound healing interventions included in systematic review.
**Wound type**

*Acute experimentally created wounds*

There was a range of effect sizes for experimentally created acute wounds (0.13 to 0.82). This category could be considered the most diverse due to the different ways wounds were created (punch biopsy, tape-stripping, blister, and laser ablation). Within this category two studies looked at the effects of relaxation (Gouin et al. 2008; Robinson et al., 2015), one looked at social support (Robles, 2007), two looked at expressive writing (Koschwanez et al., 2013; Weinman et al., 2008), and one study investigated placebo effects (Vits et al., 2013).

Two studies created wounds using a tape-stripping method. Robinson et al., (2015) compared the effects of relaxation immediately before or after skin tape stripping and compared a control group who read quietly. Regardless of its timing, relaxation improved skin barrier recovery compared to the no relaxation group with a moderate effect size. Robles (2007) also used a tape stripping method to investigate the effects of social support on skin barrier recovery prior to a stress task. There were no differences in skin barrier recovery between those that did or did not receive support but it is questionable whether the intervention helped to buffer stress in this study.

Two randomised trials investigated the benefits of expressive writing on punch biopsy wound healing (Koschwanez et al., 2013; Weinman et al., 2008). Both of these studies used the same intervention protocol, and implemented the intervention two weeks prior to the 4mm punch biopsy wound on the upper inner arm. Both studies indicated that expressive writing, in comparison to writing about time management, was beneficial for healing with similar large effect sizes 0.77 and 0.82.

The only study on blister wounds did not report a significant difference between the relaxation group and control group (asked to read quietly; Gouin et al., 2008). The authors attributed the null findings to floor effect in stress because the blister creation caused little stress and hence the relaxation intervention did not buffer stress.
Finally, one study created an acute wound using laser ablation to investigate the use of a placebo to improve healing (Vits et al, 2015). There was no significant difference in healing between participants who were told they had a non-active gel on their wounds and those who were told they had an active “wound gel” (in reality the same non-active gel) on their wounds. It could be that this type of wound is not affected by psychological interventions, or that a placebo intervention is not a useful psychological intervention for healing in general, or the study design had some weaknesses. Overall these results largely support the use of psychosocial interventions to improve the healing of acute experimental wounds, although more studies are needed especially for laser ablation and blister wounds.

**Surgical wounds**

All five studies on surgical wounds reported positive effects of psychological interventions on healing, with effect sizes from 0.53 to 1.89. Of these studies, one study investigated the impact of empathetic centred care compared to standard treatment (Pereira et al., 2016). This intervention was implemented one month prior to a surgical procedure (inguinal hernia repair, haemorrhoidectomy, pilonidal sinus resection) and focused on validation of patient concerns about surgery during a one-off 15 minute interview. There was a significant improvement in healing in the intervention group.

Another study compared the effects of two intervention groups to usual care (Ginandes et al, 2003). This study assessed the impact of either adjunctive support or hypnosis and found that both interventions had faster surgical healing compared to usual care. Patients who received hypnosis had the best outcomes. Similarly, Rao et al. (2008) compared two different types of interventions; (1) a yoga based relaxation intervention incorporating breathing exercises and attention diversion to cope with day-to-day stressful events and (2) supportive sessions focusing on education, social support and shoulder rehabilitation exercises. The active stress reduction component (yoga-based relaxation) was significantly more helpful than supportive sessions.
Other studies found that relaxation interventions were helpful for patients undergoing surgery. Broadbent et al. (2012) and Holden-Lund (1988) both conducted studies investigating the effects of relaxation on recovery from cholecystectomy surgery in comparison to usual care. These two studies had similar effect sizes (0.61 and 0.71). Overall these studies suggest that the healing of surgical wounds can be improved by psychosocial interventions.

**Burns**

Two studies looked at the benefits of distraction interventions for wound healing, both of which were conducted in a clinical context with children with burn injuries by the same research group (Brown, et al., 2014; Miller et al, 2011). The effect sizes found were 0.67 and 0.40 respectively. Although both studies showed better healing with multi-modal distraction compared to standard distraction techniques, only one had statistically significant results (Brown et al, 2014) and the studies used slightly different ways to assess healing (days to 95% re-epithelialisation versus days to re-epithelialisation).

**Fractures**

Two studies investigated whether psychosocial interventions could impact fracture healing, finding effect sizes of 0.34 and 3.21. In the first study (Ginandes & Rosenthal, 1999) a non-significant trend for improved healing was reported following hypnosis in comparison to usual care. In the second study (Oswal et al., 2011) a yogic prana energization technique was compared to usual care. The intervention involved six steps that progressively deepened internal awareness intended to develop voluntary mastery over the body’s subtle energy system. It also included breathing techniques and muscle relaxation. The results showed that participants in the intervention group had significantly faster healing.

**Stomach ulcers**

Two studies investigated the influence of psychosocial interventions on stomach ulcer healing and both had positive results. One study used cognitive behavioural strategies to alter
perceptions about healing of peptic stomach ulcers in conjunction with antibiotic treatment (Wu et al., 2012). This trial found a significant positive effect on healing compared to usual care with an effect size of 0.55. Unfortunately the paper did not provide any further description of what the intervention involved. The other study investigated the effects of a stress management programme on healing (Han, 2002). It found a significant positive effect on healing with an effect size 0.57. The programme included muscle relaxation, biofeedback training, as well as cognitive and emotional management. This was compared to a muscle relaxation programme where participants did not have one-on-one sessions, but were asked to listen to a 15 minute audio recording.

**Chronic leg ulcers**

Two studies investigated the effects of different interventions on chronic leg ulcer wounds with mostly positive results. Edwards et al.’s study (2009) compared participants assigned to a socially supportive wound care group to those assigned to individual home visits from nurses. Healing was assessed via percentage reduction in ulcer area compared to baseline, as well as by absolute ulcer area at each time point. There was a non-significant trend for a difference in percentage reduction in ulcer area at 24 weeks, but a significant difference in the absolute ulcer area with an effect size of 0.36 in favour of the social support group. At 24 weeks there was also significantly less oedema, venous eczema, and sloughy tissue in social support group wounds. The second study (Rice et al., 2001) investigated the effects of a biofeedback-assisted relaxation training intervention compared to usual care. The intervention had significant positive effects on ulcer reduction and an effect size of 0.87.

**Intervention types**

The studies can be categorized into ten broad areas representing different types of psychological interventions: relaxation (5 studies), social support (3 studies), yoga (2 studies), hypnosis (2 studies), expressive writing (2 studies), distraction (2 studies), empathetic care (1 study), stress management (1 study), cognitive behavioural therapy (1 study), and placebo (1 study). One
study compared two different types of interventions (social support and hypnosis) to usual care (Ginandes, Brooks, Sando, Jones & Aker, 2003).

Overall, it is difficult to compare specific interventions to one another given the variance in the type of wound studied and the overlapping content of many of the interventions. However, four of the five relaxation interventions, showed significant benefits for wound healing with effect sizes ranging from 0.47 to 0.87. Both expressive writing interventions had significant benefits for wound healing (effects sizes of 0.77 and 0.82).

Of the three interventions using social support, one was not significant (Robles, 2007), although the intervention may not have increased perceived social support. Edwards et al (2009) reported a significant effect for ulcer area at 24 weeks (but not for area expressed as a percentage reduction from baseline). Finally, Ginandes et al., (2003) reported a benefit in wound healing that although clinically significant was not statistically significant.

**Moderating factors**

As the effect sizes reported vary considerably, it is important to consider other moderating factors that may influence wound healing. These are discussed below.

*Participant age and gender*

There was a range of age groups studied. Two studies were conducted with children, both of which used a specific distraction device for burns. This may not be a suitable intervention for older patients or may need to be adapted for different wound types. Three studies were conducted with older adults (over 60 years), seven were conducted with adults (average age ranged between 34 and 52 years) and five were conducted with students (average age ranged between 22 and 30 years). Two studies were conducted with only male participants and two studies were conducted with only female participants. The remaining studies included both males and females. Ten studies controlled for age and gender in analyses, all finding that these variable were not associated with healing.
Based on the effects sizes, there do not appear to be any obvious differences in effects based on age group or gender.

**Intensity and duration of interventions**

The intensity and duration of each intervention is reported in Table 1. Several studies employed short single session interventions (Pereira et al., 2016; Robinson et al., 2015; Robles, 2007), whereas other studies took place over a number of weeks or months. Experimentally created wounds tended to include brief interventions, or were more intensive (sessions closer together) compared to other wound types where the intervention continued over a longer period. The duration of the intervention in each study partially reflects the healing period for each wound type. Interventions for ulcer wounds tended to take place over the longest period and ulcers were the slowest kinds of wounds to heal.

Two fairly comparable studies are Broadbent et al., (2012) and Holden-Lund (1988), who used similar relaxation interventions for cholecystectomy patients. Broadbent et al., (2012) had a longer intervention period (7 days after surgery compared to 3 days) and had a slightly smaller effect size (0.61 compared to 0.71). However, Holden-Lund (1988) supervised intervention delivery every day while in hospital whereas the patients in Broadbent et al.’s study were given the tape to listen to on their own, with reported compliance ranging from 0 to 15 times in the days after surgery. This suggests that not only is the duration of the intervention important but also the style of delivery.

**Compliance**

Compliance to intervention protocol was reported in most studies. Of the studies 19 included in the review 13 studies reported that they did not have any issues with compliance with the psychosocial intervention. As noted above, Broadbent et al (2012) repoted variable compliance. One other study reported issues with non-compliance (Rice et al. 2001), where participants were required to complete relaxation a minimum of five out of seven days (71% days practiced). On average participants practised relaxation just under 6 days a week (83% days practised). One
participant was replaced due to non-compliance. This study did not report if compliance was associated with healing. Six studies did not report compliance to audio interventions.

**Timing of intervention**

Three studies implemented the intervention prior to wounding. Two of these studies investigated expressive writing (Koschwanez et al., 2013; Weinman et al., 2008) and both had significant effects on healing of a punch biopsy wound. One study used empathetic patient centred care (Pereria et al., 2016) prior to surgery and found that this brief intervention had a significant effect on healing. Effect sizes ranged from 0.56 to 0.82. Eleven studies implemented the intervention after the wound had been sustained. The majority of these studies implemented the intervention afterwards due to the nature of the wounds (burns, fractures, ulcers). Two of these studies were conducted with experimentally created acute wounds (Gouin et al., 2008; Robles, 2007) and both had non-significant results. The effect sizes of studies that were conducted after wounding were more variable and ranged from 0.15 to 3.21.

Five studies (four conducted with surgical wounds) were conducted both before and after wounding (in preparation for surgery and during recovery). All of these were effective in promoting wound healing. The remaining study (Robinson et al., 2015) specifically compared whether there was a difference in skin barrier recovery if relaxation was performed immediately prior to or immediately after tape stripping, finding both were effective compared to a control group. The effect sizes of studies that were conducted both before and after wounding ranged from 0.47 to 1.89. It therefore appears that interventions may be effective either before or after wounding, but more research is needed on timing of delivery.

**Assessment of wounds**

Healing was assessed in a number of different ways using subjective and objective methods. Ten studies made subjective assessments of healing. These included ratings from specialists of whether or not the wound was healed based on pre-determined measures (Wound Assessment...
Inventory (WAI); Pressure Ulcer Scale for Healing (PUSH)) or the amount of time until a wound was classed as healed. Ten studies used objective measures of healing. Five of these studies assessed the area of the wound using devices (High Resolution Ultra Sound (HRUS) scanner; Visitrak) or calculated the area of the wound from photographs. Three studies used transepidermal water loss to assess skin barrier recovery after tape stripping or after a blister wound. One study assessed hydroxyproline deposition within the wound as an indicator of collagen synthesis, which occurs during the proliferative phase of healing. The effect sizes using subjective ratings of healing ranged from 0.34 to 3.21. The effect sizes using objective methods ranged from 0.13 to 0.77.

Mediation - stress and mood

Many studies proposed that the effects of psychosocial interventions on wound healing were mediated by reductions in stress or negative mood and subsequent reductions in stress hormones. Of the 19 studies included in this review, four studies assessed stress using a psychometric tool and two studies assessed a physiological measure of stress (cortisol, alpha amylase, physiological arousal). A further three studies used both self-reported and physical measures of stress. Nine out of 19 assessed anxiety (6 studies) and/or depression (5 studies). The measures are summarised in Table 2.

Only two studies investigated whether changes in stress were associated with wound healing. Gouin et al. (2008) found that although participants did not benefit from relaxation, lower levels of cortisol at the time of wound were associated with faster healing 4 days later. Broadbent et al., (2013) reported that as a result of a relaxation intervention self-reported stress decreased, but decreases in stress did not mediate faster wound healing. For the other studies, one study measured self-reported stress only at baseline (Robinson et al., 2015), two studies reported significantly faster healing in intervention groups as well as significant reductions in physiological stress levels (Holden-Lund, 1988) and physiological and self-reported stress levels (Han, 2002). Three studies reported significantly faster healing in the intervention group but no significant changes in self-reported stress
(Koschwanez et al., 2013; Weinman et al., 2008) or changes in both self-reported stress and physiological stress responses (Brown et al., 2014).

For those studies that assessed mood, two studies (Gouin et al., 2008; Robles, 2007) found no significant changes in mood as a result of the intervention and no significant difference in healing between groups. Four studies reported significantly faster healing in intervention groups and significant changes in mood (Brown et al., 2014; Holden-Lund, 1988; Pereira et al., 2016; Wu et al., 2012). Finally, three studies (Edwards et al., 2009; Koschwanez et al., 2013; Weinman et al., 2008) reported significantly faster healing in the intervention group but no significant changes in depression. These findings provide mixed evidence that psychosocial interventions are mediated via changes in mood state and stress.
Table 2: Physiological measures of stress, self-reported stress and self-reported mood for each study

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Physiological stress measure, time points measured</th>
<th>Outcome</th>
<th>Self-reported stress measure, time points measured</th>
<th>Outcome</th>
<th>Mood measure (Anxiety, depression), time points measured</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimentally created wounds</td>
<td></td>
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<tr>
<td>Gouin, Kiecolt-Glaser, Malarkey &amp; Glaser, 2008</td>
<td>Salivary cortisol (ug/dl) Measured 30 minutes before blister wounding and every hour for 15 hours after blister wounding</td>
<td>Lower cortisol production during blister wounding significantly associated with greater likelihood of being healed on day 4 ( p = .016 ).</td>
<td>-</td>
<td>-</td>
<td>Beck anxiety index (BAI) Measured 15 minutes before blister wounding</td>
<td>No significant association with amount of days to heal and anxiety</td>
</tr>
<tr>
<td>Koschwanez, Kerse, Darragh, Jarrett, Booth &amp; Broadbent, 2013</td>
<td>-</td>
<td>Percieved stress scale (PSS) Measured 3 weeks before wounding, the day of wounding and 6 weeks after wounding.</td>
<td>No significant differences between groups in perceived stress</td>
<td>Beck depression index (BDI) Measured 15 minutes before blister wounding Geriatric depression scale (GDS) Measured 3 weeks before wounding, the day of wounding and 6 weeks after wounding.</td>
<td>No significant association with amount of days to heal and depression</td>
<td></td>
</tr>
<tr>
<td>Robinson, Jarrett &amp; Broadbent, 2015</td>
<td>-</td>
<td>Percieved stress scale (PSS-10) Measured 30 minutes before tape stripping procedure</td>
<td>No significant association of perceived stress with skin barrier recovery</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Robles, 2007</td>
<td>Salivary cortisol (ug/dl) Measured before and after tape stripping procedure and 10, 30, 45, 60, 75 and 90 minutes after stress task</td>
<td>No significant difference between groups that completed the stress task and did or did not have social support prior to stress task</td>
<td>Single question asking about perceived stress Measured after stress task</td>
<td>No significant difference between groups that completed the stress task and did or did not have social support prior to stress task in perceived stress</td>
<td>State anxiety inventory (STAI) Measured after skin barrier disruption and after stress task</td>
<td>No significant difference between groups that completed the stress task and did or did not have social support prior to stress task in anxiety</td>
</tr>
<tr>
<td>Weinman, Ebrecht, Scott, Walburn &amp; Dyson 2008</td>
<td>-</td>
<td>Percieved stress scale (PSS) Measured 2 weeks before wounding, the day of wounding and 2</td>
<td>-</td>
<td>General Health Questionnaire (GHQ-12) Measured 2 weeks before wounding, the day of wounding and 2</td>
<td>No significant differences between groups in depression</td>
<td></td>
</tr>
</tbody>
</table>

58
<table>
<thead>
<tr>
<th>Study</th>
<th>Methodology</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surgical wounds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broadbent, Kahokehr,</td>
<td>Perceived stress scale</td>
<td>Significant reduction in stress in the intervention group compared with</td>
</tr>
<tr>
<td>Booth, Thomas, Windsor,</td>
<td>(PSS-4)</td>
<td>the control group controlling for baseline stress and age, ($p = .048$)</td>
</tr>
<tr>
<td>Buchanan, Wheeler, Sammour &amp; Hill,</td>
<td></td>
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<tr>
<td>2012</td>
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<tr>
<td><strong>Perceieved stress scale</strong></td>
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<tr>
<td>Holden-Lund, 1988</td>
<td>Urinary cortisol (mcg/100ml per</td>
<td>Significantly lower levels of cortisol in the intervention group 1 day</td>
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<td></td>
<td>creatinine)</td>
<td>after surgery ($p &lt; .05$).</td>
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<td></td>
<td>Measured 2 days before surgery,</td>
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<td></td>
<td>and every day for 3 days after</td>
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<td></td>
<td>surgery</td>
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<tr>
<td><strong>Burns</strong></td>
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<tr>
<td>Brown, Kimble, Rodger,</td>
<td>Salivary cortisol (ug/dl)</td>
<td>No significant differences between groups in salivary cortisol</td>
</tr>
<tr>
<td>Ware &amp; Cuttle, 2014</td>
<td>Measured on arrival to the clinic,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>after dressing change, 10 minutes</td>
<td>No significant differences between groups in child trauma</td>
</tr>
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<td></td>
<td>after dressing change for 1st 3</td>
<td></td>
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<tr>
<td></td>
<td>dressing changes and 3 months</td>
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<td></td>
<td>post reepithelialisation</td>
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<td></td>
<td>Child Trauma Screening</td>
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<td></td>
<td>Questionnaire (CTSQ)</td>
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<td></td>
<td>Measured after 1st dressing</td>
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<td></td>
<td>change and 3 months post</td>
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<td></td>
<td>reepithelialisation</td>
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<td></td>
<td>Visual Analogue Scale-Anxiety</td>
<td>Significantly lower anxiety ratings in the intervention group prior to</td>
</tr>
<tr>
<td></td>
<td>(VAS-A)</td>
<td>the first dressing change ($p = .022$) and after the first dressing</td>
</tr>
<tr>
<td></td>
<td>Measured on arrival to clinic,</td>
<td>change ($p = .051$). At the second and third dressing removals average</td>
</tr>
<tr>
<td></td>
<td>after intervention and pre</td>
<td>anxiety scores were at least one point lower in the intervention group.</td>
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<tr>
<td></td>
<td>dressing change, after dressing</td>
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<td></td>
<td>change and after subsequent</td>
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<td></td>
<td>dressing changes</td>
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<td></td>
<td>Salivary alpha amylase (U/ml)</td>
<td>No significant differences between groups in salivary alpha amylase</td>
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<tr>
<td></td>
<td>Measured in the waiting</td>
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<td></td>
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<tr>
<td>Condition</td>
<td>Methods</td>
<td>Measurements</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Stomach ulcer</td>
<td>Wu, Guo, Gao, Kang, Guo, Jiang, Chen, Liu &amp; Li, 2012</td>
<td>Self rating anxiety scale (SAS) Measured before and after intervention</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Self rating depression scale (SDS) Measured before and after intervention</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Symptoms of stress scale (SOS) Measured before and after intervention.</td>
</tr>
<tr>
<td>Han, 2002</td>
<td>Physiological stress reaction with a biofeedback instrument, which included galvanic skin temperature, muscle tension level, heart rate, respiratory rate and skin conductance level. Measured before and after intervention.</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Intervention group had significantly lower muscle tension ($p &lt; .001$), respiratory rate ($p &lt; .001$), skin conductance ($p = .05$) and almost significantly lower heart rate ($p = .06$) after the intervention</td>
<td>-</td>
</tr>
<tr>
<td>Chronic ulcer</td>
<td>Edwards, Courtney, Finlayson &amp; Shuter, 2009</td>
<td>Geriatric depression scale (GDS) Measured before intervention and then 12 and 24 weeks after starting intervention</td>
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</table>
Discussion

This review identified 19 randomized controlled trials that investigated the effects of psychological interventions on wound healing in humans. Overall the results provide cautious support that psychological interventions have positive effects on wound healing. The research reflects a range of different wound types in both clinical and experimental settings and a range of psychosocial interventions. Due to this heterogeneity, more research needs to be conducted to support these findings. Only a small number of studies have been conducted for each type of psychosocial intervention and because these findings span various wound types it is difficult to identify what type of intervention would be most appropriate for different wounds. There was possible publication bias in that small negative studies were not represented, and several studies included had low quality assessments. The findings need to be considered within the current context of the limited literature identified to date.

Most research has been performed with acute rather than chronic wounds. Experiments on small experimental wounds in healthy individuals may offer initial evidence for interventions that can be further tested in clinical settings. For example, two studies conducted with punch biopsy wounds showed beneficial effects of expressive writing. However, since the time period of this review, one study has been published suggesting that expressive writing may not be effective for bariatric surgery patients (Koschwanez et al., 2017). Research with experimentally created wounds could focus on further investigating different ways to deliver interventions and potential mechanisms to help understand how the intervention may have an optimal impact when translated to other settings.

For all types of wounds except laser ablation, there was at least some support for the effectiveness of psychological interventions on healing. Evidence was consistently strong for surgical wounds and this category contained the most studies. Various kinds of surgery were represented, and relaxation, supportive interventions, hypnosis and yoga all positively influenced healing. Surgical
patients may have had the highest stress and anxiety levels and therefore the interventions had more potential to reduce high stress levels. Stress and anxiety may also be elevated in patients with other types of clinical wounds. Both studies looking at psychosocial interventions for chronic leg ulcers found at least some positive results. Similarly, the two studies investigating psychosocial interventions for stomach ulcers found positive results, but the low study quality rating should be noted. The interventions used with fracture healing indicated that yoga may be useful to aid healing but not hypnosis. Finally the studies looking at burn wounds used a specific distraction intervention for young children during burn dressing changes and only one was significant. More research is needed to determine whether specific interventions are better suited for a particular patient group or wound type.

In terms of intervention types, relaxation showed the most benefits for wound healing and this category included more trials than any other category. Social support showed tentative evidence for benefits in wound healing but the interventions differed in the type of support offered. Yoga, empathic care and expressive writing interventions all had significant effects on wound healing. The remaining interventions using hypnosis, distraction, stress management, cognitive behaviour therapy and placebo had mixed results and more high quality studies conducted by independent research groups are needed.

It is difficult to determine which aspects of psychosocial interventions are beneficial because many of the interventions had overlapping content. For example, brief empathetic care had many similarities to formal social support. Similarly, relaxation interventions incorporated a number of different strategies (deep breathing, guided imagery, biofeedback, progressive muscle relaxation). Yoga also included relaxation techniques. Conversely, studies included under the broad category of social support differed markedly from each other. Each intervention considered a different aspect of social support (peer support, stranger support, and formal support) and none included support from
family members. Further research is needed to investigate which types of support should be offered, when, and to whom.

The findings of this review are similar to other reviews on the influence of psychological interventions on immune parameters (Miller & Cohen, 2001; Wahbeh et al., 2009) and surgical outcomes (Devine, 1992; Johnston & Vogele, 1993; Nelson et al., 2013). These reviews also found evidence for the use of relaxation, psychosocial interventions, and expressive writing. Miller and Cohen (2001) highlighted that despite some of the mixed findings for improvements using psychological interventions, many studies had methodological issues and the results should not be interpreted in a way that suggests that psychological interventions are of limited use.

More research is needed to refine how each intervention should be implemented (at what time point, by whom, period of intervention, and frequency of sessions). There may be advantages to intervening prior to wounding versus after wounding. For example, surgery causes anxiety (Badner, Nielson, Munk, Kwiatkowska & Gelb, 1990), and research has found that reducing anxiety prior to surgery leads to better surgical and psychological outcomes (Lin & Wang, 2005). However, patients experience the most pain, boredom and sleep interruption during recovery and may have more time to complete interventions after surgery.

The method of assessing healing is another important consideration. The majority of studies used subjective ratings. Assessment of wound healing needs to be standardized and ideally measured both subjectively and objectively to get a valid and clinically relevant measure and to allow wound healing to be compared across studies.

At present there is insufficient evidence to show the mechanisms via which psychosocial interventions lead to improved healing. Although interventions are thought to reduce stress and negative mood and cause reductions in stress hormones, many studies did not measure these variables. Future studies should measure both changes in mood/stress and changes in the HPA and
SAM systems. Interventions may have an impact on healing via pathways other than stress reduction. For example, research looking at the role of stress in the development and prognosis of peptic stomach ulcers indicates that stress leads to behavioural changes (reduction of sleep, poor diet, smoking, drinking) that may impede recovery via changes in stomach acid secretion (Levenstein, 1998). Similarly interventions that concentrate on the experience of pain may help wound healing by changing the way pain is perceived. Pain plays an important role in wound healing, as not only does it interact with biological mechanisms involved in healing (Widgerow & Kalaria, 2012), but also elevates levels of distress (Wallace, 1985), completing a self perpetuating loop, impeding wound healing. An active focus of future research should be on identifying the active components of interventions as well as the mechanisms by which interventions improve healing.

There are a limited number of studies on psychological interventions and wound healing and those conducted to date are diverse. One of the major challenges of consolidating this research is that different types of wounds have been studied and it cannot be assumed that all wounds follow the same healing pattern or are influenced by the same physiological and psychological processes. For example, an experimentally created biopsy wound first involves blood coagulation, followed by a much longer and more intense inflammation phase in the tissue, compared to a superficial skin wound caused by tape stripping. Tape stripping that does not break the skin is associated with less pain, and healing can be measured over minutes and hours as opposed to days and weeks. Surgical wounds take longer to heal because they involve deep tissue repair. The repair of fractured bones also involves different healing process than other tissues. Chronic wounds have different healing projection as well as a different set of factors that must be considered in the healing process. Chronic ulcers are usually associated with tissue hypoxia (Tandara & Mustoe, 2004), which impairs wound healing by prolonging the inflammatory response (Mathieu, Linke & Wattel, 2006). For
patients with diabetes impaired healing may also be associated with dysfunction in fibroblasts involved in the repair of connective tissue and repair of blood vessels (Guo & DiPietro, 2010).

**Implications for clinical practice**

Many of the interventions reviewed here could be feasibly and inexpensively incorporated into clinical settings. Psychosocial interventions have shown good effects sizes when applied to surgical patients and ulcers, especially interventions that incorporate relaxation techniques. However, caution should be applied to interventions that have not yet been tested in clinical settings. More research needs to investigate compliance with interventions and if there are robust findings across larger populations with different wound types.

**Conclusions**

The results of this review suggest that psychological interventions can improve wound healing. Although the identified interventions are not directly comparable because of the range of wounds and methodological differences, many interventions share similar components because they promote relaxation and positive coping strategies. More work is needed to assess the efficacy of different interventions on different wound types, and study the mechanisms by which interventions improve healing.
Chapter 4: The effects of relaxation before or after skin damage on skin barrier recovery: A preliminary study

Preface

Rationale

As highlighted in Chapter 3, relaxation strategies have been found to have a number of health benefits and a limited number of studies have found that relaxation can benefit wound healing. Of the studies that have been conducted, the research approaches differ in terms of the type of wound that has been studied, and the way in which healing was assessed. To date relaxation research has been conducted with surgical wounds (Broadbent et al., 2012; Holden-Lund, 1988) and chronic wounds (Rice et al., 2001). Wound healing has been assessed either by being rated by doctors, calculating the percentage of healed tissue from photographs or measured by the amount of collagen in the tissue. Despite these differences in approaches, each study found beneficial results related to wound healing. The findings are in line with literature describing the benefits of relaxation on buffering of the physiological stress response (Jacobs, 2001), reductions in pain (Kwekkeboom & Gretarsdottir, 2006), and improvements in psychological well-being which translate into specific illness and surgical outcomes (Kim, Na & Hong, 2016; Luebbert, Dahme & Hasenbring, 2001).

The literature review conducted in Chapter 3 also drew attention to other areas within the wound healing literature that have yet to be examined. A practical consideration in determining the efficacy of an intervention is understanding when it can logistically be administered and whether the timing of the intervention is influential to wound healing. In order to understand the best practical application of psychological interventions in a clinical setting, the timing of the intervention should be explored as a potential factor. The first phase of wound healing is crucial in delaying a normal healing process and changes occurring during this inflammatory phase may have implications for overall healing. For patients who are suffering from pain and distress post-surgery a relaxation intervention may be helpful in promoting healing. Although research has looked at the benefits of
relaxation strategies in a post-surgical setting, they have not measured wound healing as an outcome. Of the studies that have been conducted looking at wound healing in surgical settings the main focus of the intervention was to address stress and anxiety prior to surgery (Broadbent et al., 2012; Holden-Lund, 1988). Patients who have unscheduled surgery do not have the luxury of having a pre-surgical period to help them prepare.

The first experimental study presented here in Chapter 4 (Study 1) used a tape stripping model to investigate the effects of a relaxation intervention on wound healing. The tape stripping model has been used in previous research (Robles, 2007; Robles et al., 2009) and offers a cost-effective and controlled way of investigating the question of timing. The skin is constantly renewing itself making it an ideal way of assessing inflammatory processes, and changes to inflammatory processes over short periods of time. Any damage to the skin such as lacerations, solvents, and removal of corneocytes from the outermost layer, the stratum corneum, disrupts and reduces skin barrier function. When the skin is damaged lipid synthesis and cytokine expression occurs to initiate the healing response (Ghadially, Brown, Sequeira-Martin, Feingold & Elias, 1995). Furthermore, research has found that skin repair and cutaneous inflammatory responses are responsive to emotional stress (Al'Abadie et al., 1994; Locala, 2009). However, skin conditions associated with abnormal inflammatory responses, such as psoriasis and atopic dermatitis, have been improved by relaxation techniques (Kabat-Zinn et al., 1998; Keinan, Segal, Gal & Brenner, 1995). This indicates that relaxation influences the stress response, resulting in altered immune function, which is evident in the improvements in skin function and repair, as well as research looking at wound healing.

Study 1 is a randomised controlled trial conducted with a healthy population, designed to assess whether a brief relaxation intervention could influence skin barrier recovery. It explored whether the timing of a psychological intervention can impact recovery. It was hypothesized that participants randomised to perform relaxation either before or after the tape stripping procedure would show faster skin barrier recovery compared to those assigned to a control group.
Aims

1. To investigate whether a brief relaxation intervention incorporating deep breathing and progressive muscle relaxation could improve skin barrier recovery in a sample of healthy participants compared to a control group.

2. To explore whether the timing of the relaxation intervention (either before or after wounding) influenced skin barrier recovery.

3. To identify other psychological factors that may mediate the effects relaxation on wound healing.

Citation

**Abstract**

**Objectives:** Psychological interventions administered before wounding can reduce stress and improve healing. However, in many cases, it would be more practical for interventions to be delivered after wounding. This preliminary study investigated whether a brief relaxation intervention could improve healing when administered either before or after skin damage produced by tape stripping in comparison to a control group.

**Methods:** One hundred twenty-one healthy adults were randomised into one of three groups: a) relaxation pre-stripping group, b) relaxation post-stripping group, or c) no relaxation. Participants completed measures of stress, fatigue, relaxation, and pain. Relaxation consisted of listening to 20 minutes of guided relaxation, whereas the control condition was quiet reading for 20 minutes. Skin barrier function was measured using transepidermal water loss at baseline, immediately after tape stripping and 25 minutes later.

**Results:** Relaxation either before or after tape stripping improved skin barrier recovery compared with the control group ($F(2,92) = 3.58, p = .032, \text{partial } \eta^2 = 0.074$). Participants who took part in the relaxation intervention were significantly more relaxed and reported greater reductions in pain than the control group did 25 minutes after tape stripping. Perceived stress over the last month was not significantly related to healing.

**Conclusions:** This study showed that a relaxation intervention had a beneficial effect on skin barrier recovery regardless of whether the intervention was administered before or after wounding. Future research needs to replicate these findings in other wound types and in clinical settings, and investigate the biological mechanisms involved.
Introduction

A recent systematic review concluded that psychological stress adversely affects the immune system and impairs wound healing (Walburn et al., 2009). Furthermore, studies have shown that stress reduction interventions can reduce stress and improve wound healing. Expressive writing (writing about traumatic events) 2 weeks before 4-mm punch biopsy wounds were made to the upper arm increased the speed of healing in both young and old healthy adults (Koschwanez et al., 2013; Weinman et al., 2008). In addition, relaxation and stress management programs have been shown to improve wound healing in surgical patients. For example, cholecystectomy patients who listened to audiotapes of relaxation with guided imagery on the day before surgery and for three post-operative days had reduced anxiety, lowered cortisol levels, and reduced wound erythema compared with patients in a control group (Holden-Lund, 1998). More recently, a randomised controlled trial demonstrated that an intervention asking patients to listen to a relaxation track for 3 days before surgery and for 7 days afterward reduced stress and increased wound collagen in patients undergoing a laparoscopic cholecystectomy (Broadbent et al., 2012). Relaxation has been linked to changes in autonomic functioning and immune parameters (Critchley, Melmed, Featherstone, Mathias & Dolan, 2001; Friedman & Irwin, 1997; Lucini et al., 1997), which may explain why performing relaxation can have beneficial effects.

In all these wound healing studies, the interventions were administered before wounding or before and after wounding. The theory behind this is that psychological stress reduction before wounding may enable the body to mount a more efficient response to the initial wound. The initial phases of wound repair are considered critical to later stages. However, an intervention may be most practical to administer after a wound has occurred. Pre-surgery is a busy time for most clinics, and operations may be performed with little time to schedule relaxation training. In addition, patients will have much more time in which to perform relaxation when they are off-work after an operation. Despite the lack of interventions looking at facilitating wound healing post-surgery, there is some evidence suggesting that relaxation techniques have a positive effect on other outcomes.
A review of 21 studies concluded that post-operative relaxation and music had a positive effect on pain (Good, 1996). Given that pain has been linked to poor healing (McGuire et al., 2006), relaxation delivered after wounding may also be able to improve healing.

The aim of the current research was to investigate the effects of timing of a relaxation intervention on wound healing. We used a tape stripping model, which triggers an inflammatory immune response to the damage inflicted to the skin and provides a relevant model by which to observe wound healing inexpensively and non-invasively within a short period. Factors that affect skin healing in the laboratory may be applicable to surgical populations and people with skin conditions that are exacerbated by stress. The skin is an integral part of the immune system, protecting the body against foreign pathogens and preventing fluid loss. The act of tape stripping impairs the stratum corneum, initiating a cascade of immunological events to immediately repair damage to the skin (Gouin & Kiecolt-Glaser, 2011). However, the skin is highly innervated, and when a stress response occurs, neuroendocrine chemicals send messages to the skin that may interfere with a proficient immunological response to skin damage. In particular, psychological stress has been found to interfere with skin barrier recovery (Garg et al., 2001, Robles, 2007) and has been found to exacerbate some dermatological skin conditions (Shenefelt, 2010). Psychological stress can negatively affect permeability barrier homeostasis and stratum corneum integrity and cohesion through an increase in endogenous glucocorticoids, and these glucocorticoids act to suppress epidermal lipid synthesis leading to compromised skin barrier function and integrity (Choi et al., 2005). In addition, stress can affect the proliferation of cytokines (Tsai et al., 1994), which are necessary to help restore the skin barrier (Nickoloff & Naidu, 1994; Glaser et al., 1999; Wood et al., 1997). Psychological interventions to reduce stress either before wounding or after wounding may reduce stress hormones and their interference with skin barrier recovery. Although past research has used tape stripping to look at the relationship between stress and skin barrier recovery, no intervention studies have been conducted using this method. It is hypothesized that groups receiving
a relaxation intervention either before or after tape stripping will have improved skin repair compared with a control group.

Methods

Sample

Participants were recruited from the local community and university campus through flyers, e-mail, and online advertisements. Participants had to be older than 16 years and provide written informed consent. Participants were excluded if they had allergies to tape or adhesives, had an inflammatory dermatological condition, or were taking medication affecting the immune system. Data collection took place between November 2013 and May 2014 between 8 AM and 4 PM. Ethics approval was obtained from The University of Auckland Human Participants Ethics Committee.

Previous research found the effect of relaxation on wound healing to be moderate to large ($d = 0.60$; Broadbent et al., 2012). Using an effect size of $f = 0.30$, with power set at 0.80 and a two-tailed significance level of $\alpha = .05$, the total sample size required for an analysis of covariance (ANCOVA) analysis with three groups was calculated using G*Power to be 111 (37 participants per group; Faul, Erdfelder, Lang & Buchner, 2007). To account for attrition and exclusions, a total of 40 participants per group were recruited.

Procedure

Participants took part in a 90-minute session at The University of Auckland Clinical Research Centre in a ventilated room, where the temperature was controlled by the central university heating system (average temperature, 23°C; average humidity, 56%). These conditions were consistent with the guidelines for operating the TEWLmeter probe recommended by the manufacturer. Participants were asked not to drink caffeinated drinks for 1 hour before the session or shower or exercise for half an hour before the session. Participants were also instructed not to apply moisturizer to their arms on the day of the session. At the beginning of the session, participants completed a baseline questionnaire relating to health behaviour, stress, mood, and pain.
They were randomly allocated to one of three groups: control, relaxation pre-stripping group or relaxation post-stripping group. Randomisation was performed by a researcher uninvolved in this project using a random number generator. When the participant arrived for the session, the researcher opened an envelope after the baseline questionnaire was completed revealing the group to which the participant was allocated. The researcher delivering the intervention and performing the tape stripping was therefore not blinded to the condition of the participant.

Those in the relaxation pre-stripping group were instructed to listen to a 20 minute guided relaxation track before tape stripping. Participants in the relaxation post-stripping group and control groups were told that their skin needed to acclimatize to the surroundings for 20 minutes before tape stripping. During this time, they were offered a magazine to read. The researcher left the room for this 20 minute period. Upon returning, the researcher asked the participants to complete a questionnaire and then baseline skin measurements were obtained. Skin barrier disruption was performed using a standardised tape stripping procedure for all groups. After this, participants completed another brief questionnaire on pain. Participants in the relaxation post-stripping condition then listened to the 20 minute relaxation track, and participants in the relaxation pre-stripping and control groups were told that they need to wait 20 minutes to see how their skin recovered, during which they were asked to read magazines. The researcher waited again in the adjacent room during this 20 minute period. When the researcher re-entered the room, participants were asked to complete a final questionnaire and then a follow-up measure of skin recovery was measured. All participants were given a $10 voucher as compensation for their time. Participants were only required to come to the clinic for one appointment. There were no other follow-up sessions.

Relaxation intervention

The relaxation intervention was pre-recorded and played as an audio file through a set of headphones. For the 20 minute session, the researcher dimmed the fluorescent lights to make the room more relaxing. The instructions on the file asked participants to close their eyes and guided
them through deep breathing and progressive muscle relaxation while gentle music was played in
the background.

Measures

Tape stripping and skin barrier recovery

Tape stripping is a commonly used procedure in dermatological research to disrupt the skin
barrier (Fluhr, Feingold & Elias, 2006). Baseline skin barrier function was measured by obtaining
baseline readings of transepidermal water loss (TEWL) using TEWL meter (Courage Khazaka, Köln,
Germany), which measures evaporation of water from the skin (evaporation rate in grams per hour
per metre squared). The TEWL meter probe measures the density gradient of the water evaporation
from the skin indirectly by two pairs of sensors (temperature and relative humidity) inside the
hollow cylinder. TEWL indicates the skin’s ability to prevent water loss from the epidermis.

Three 1 cm\(^2\) areas on the inside of the forearm of the left arm were marked. These areas
were adjacent to each other 1 cm below the elbow crease. A 1 cm\(^2\) non-disrupted control site was
marked 1 cm below the test sites. The TEWL meter probe sat in a probe heater set to 34°C before use
to ensure that it was as close to skin temperature as possible to obtain a reliable reading. A baseline
measure of all four sites was taken using the TEWL meter. The participants were instructed to keep
their arm flat and still on a cushion on a desk while the measurement was taken. The TEWL meter
measured each site for 1 minute, taking a measurement each second. The three test sites were then
dry shaved with a disposable razor to ensure that no hair was pulled out during tape stripping.
Standard tape (Scotch Clear Packaging Tape, 3M) was applied 20 times to the three test areas (a
pilot study undertaken before the commencement of this study found that all six participants
required at least 20 strips before the skin was elevated by 15 g/h/m\(^2\)). Each strip was gently pushed
on to the arm and removed. After 20 strips, the first site was tested to determine whether the skin
barrier had been disrupted to a minimum of 15g/h/m\(^2\) above baseline. If not, another 10 strips were
applied to the test area and it was tested again. Tape stripping stopped after 40 strips of tape had
been applied or the skin was elevated by 15 g/h/m\(^2\). Higher TEWL indicates that more water loss is
occurring through the skin and skin barrier function is decreased. Faster return to baseline TEWL levels indicates faster healing. A measurement for each site including the control site was taken to determine the elevated level. For the rest of the session, the participants kept their arm uncovered and were instructed not to touch the area. At the end of the session, the skin was measured again using the TEWLmeter. The 25 minute period was chosen because we wanted to see if relaxation had an immediate difference on recovery. Results from previous studies indicate that the greatest increase in healing occurs within the first hour (Robles, 2007; Robles et al., 2009).

**TEWL analysis**

Twenty consecutive measurements with a standard deviation (SD) less than 0.5 were averaged to give an overall TEWL reading for each site and time point. Skin barrier recovery was computed as a percentage based on the formula used in several studies: Recovery = \( \frac{(\text{TEWL}_{\text{post-tape stripping}} - \text{TEWL}_{25 \text{ min follow-up}})}{\text{TEWL}_{\text{post-tape stripping}} - \text{TEWL}_{\text{baseline}}} \times 100\% \) (Robles, 2007; Robles et al., 2009). Greater values reflect greater skin barrier recovery.

There was a large variation in TEWL readings which may have been due to the room being ventilated rather than climate controlled (Miteva, Richter, Elsner & Fluhr, 2006). To ensure only valid data were included in the analysis, careful and systematic data screening was conducted. For inclusion, participants had to have complete readings at all three time points (\( N = 119 \)). Exclusion criteria for sites were as follows: interference with the site (scratching, \( N = 1 \)), recovery more than 200% (similar to previous research (Robles, 2007; \( N = 3 \)), baseline reading showed high TEWL (2 SDs above the mean; \( \text{Mean} = 15.58, \text{SD} = 6.44; N = 2 \)), skin was not sufficiently impaired (<5g/h/m²; \( N = 9 \)), and skin was damaged too much (outside 2 SDs of elevation; \( \text{Mean} = 23.69, \text{SD} =20.66; N = 4 \)).

If the readings of the remaining stripped sites were all within 10g/h/m², all sites were averaged. Otherwise, the closest two sites were averaged as per Robles (2007). If the remaining sites were not within 30 g/h/m² of each other, the readings were excluded because this indicates that a reliable reading was not obtained (\( N = 7 \)). After exclusion, there were 29 participants in the control group, 30 in the relaxation pre-stripping group, and 34 in the relaxation post-stripping group with
reliable skin recovery readings (see Figure 3). There were no differences between participants who
had their data excluded compared with those who were included in ethnicity, age, sex, room
conditions, or number of tape strips applied.
Responded to study advertisement \((N=142)\)

Excluded \((N=21)\)
- Decided not to participate \((N=12)\)
- Could not commit to session \((N=5)\)
- Excluded due to skin condition \((N=3)\)

Randomized \((N=121)\)

Allocated control group \((N=40)\)
- 20 minutes of quiet reading
- Tape stripping and TEWL assessment
- TEWL assessment

Allocated relaxation pre-stripping group \((N=40)\)
- 20 minutes of quiet reading
- Tape stripping and TEWL assessment
- TEWL assessment

Allocated relaxation post-stripping group \((N=41)\)
- 20 minutes of quiet reading
- Tape stripping and TEWL assessment
- TEWL assessment

Analyzed for relaxation and pain \((N=40)\)
Analyzed for skin barrier recovery \((N=29)\)

Excluded \((N=11)\)
- skin not elevated or elevated by less than 5 g/h/m\(^2\) \((N=4)\)
- skin elevated by more than 65 g/h/m\(^2\) \((N=1)\)
- remaining sites were not within 30 g/h/m\(^2\) (no reliable reading; \(N=3\))
- incomplete data \((N=1)\)
- recovery over 200% \((N=2)\)

Figure 3. CONSORT flow diagram.
Demographic and psychological measures

Participants were asked about their age, weight, height, ethnicity, and education level. Health-related data were collected regarding alcohol consumption, smoking status, exercise regularity, diet, and sleep. Participants were asked to rate their alcohol consumption over the past 3 months from 1 (“never”) to 6 (“everyday”). On days they did drink, they were asked to rate how many drinks they had ranging from 1 (“0 drinks”) to 7 (“12 or more drinks”). Participants were asked to rate how often they did physical activity for 30 minutes over an average week from 1 (“never”) to 8 (“everyday”). They were asked to rate their diet over the past week from 1 (“very poor”) to 5 (“very good”). Participants were asked to estimate the number of hours they slept on average per night over the last month and rate the quality of this sleep on a scale from 1 (“very poor”) to 5 (very good”). Finally, participants were asked the number of hours of sleep they had over the past 24 hours.

The 10-item Perceived Stress Scale (PSS; Cohen & Williamson, 1988) was used to determine how much participants felt their lives were unpredictable, uncontrollable, and stressful at baseline. Respondents were asked to indicate how often they felt a certain way over the last month on a scale from 0 (“never”) to 4 (“very often”).

Participants were asked to complete visual analogue scales for relaxation and pain. Participants were asked to mark on a 100 mm line how they were currently feeling with “not relaxed at all” and “extremely relaxed” as the two anchors. For pain, the anchors were “no sensation of pain” and “most sensation of pain imaginable.” Participants were given these scales at four time points: baseline, after the first session, after tape stripping, and follow-up.

Data analysis

All data were analyzed using the Statistics software package “IBM SPSS 19.0 for Windows.” To assess skin barrier recovery between groups, Quade’s rank ANCOVA (Quade, 1967) was performed, which is a non-parametric version of an ANCOVA. Pre-stripping and post-stripping skin barrier function measurements were transformed to ranks by ranking each pre-stripping and post-
stripping measurement. This has been used in previous studies where assumptions of normality were violated (Bonate, 2000; Thurber, Bodenhamer-Davis, Johnson, Chesky & Chandler, 2010). To identify covariates, correlational analyses were performed on the final sample after exclusions. Analysis was conducted with and without covariates. To determine whether the relaxation intervention had an impact on psychological variables, mixed analyses of variance or Kruskal-Wallis tests were performed to look at the changes over time and between groups for self-rated feelings of relaxation and pain.

Results

Demographics

A total of 121 people participated in this research, aged between 18 and 82 years (Mean = 23.98, SD = 9.89 years), consisting of 87 women and 34 men. Most of the sample were New Zealand Europeans (N = 57; 47%), and other ethnicities in the sample were Chinese (N = 18; 15%), Indian (N = 14; 12%), Korean (N = 11; 9%), Japanese (N = 9; 7%), Māori (N = 4; 3%), Middle Eastern (N = 4; 3%), and other European (N = 4; 3%). Most participants rated their education level as having completed a university degree (N = 60; 51%) or had completed high school and were currently studying toward a degree (N = 51; 41%). The remaining sample had completed a trade or technical certificate (N = 7; 6%) or a university diploma (N = 3; 2%). There were no significant differences in demographics, health behaviours, or perceived stress between groups at baseline (p > .05).

Table 3 shows that there were no significant differences between groups in demographic or psychological variables at baseline. There were no significant differences between groups in the mean number of strips applied or elevation in TEWL after stripping (p > .05). Wilcoxon signed rank tests showed that the control site (not stripped) was elevated significantly less than the disrupted sites, showing that the tape stripping did impair skin barrier (Z = −7.96, p < .001, r = −0.86). Repeated-measures analysis of variance found that there were no significant changes over time in TEWL in the control site (p > .05). There were no significant differences in the control site readings between groups at baseline, midpoint, or follow-up (p > .05).
Table 3. Demographics and baseline characteristics in the three experimental groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (N=40)</th>
<th>Relaxation pre-stripping (N=40)</th>
<th>Relaxation post-stripping (N=41)</th>
<th>p</th>
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<tbody>
<tr>
<td><strong>Demographic variables</strong></td>
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<tr>
<td>Gender- Females (N, % female)</td>
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<td>Age (Mean, SD)</td>
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<td><strong>Health Behaviours</strong></td>
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<td>BMI (Mean, SD)</td>
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<tr>
<td>No of times alcohol consumed over past three months</td>
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<tr>
<td>Exercise per week</td>
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<tr>
<td>Sleep per night (hours)</td>
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<tr>
<td>Quality of sleep (rating out of 5; 1=very poor, 5=very good)</td>
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<tr>
<td>Diet (rating out of 5; 1=very poor, 5=very good)</td>
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<tr>
<td>Perceived Stress Scale (PSS)</td>
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<tr>
<td>VAS relaxed</td>
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<tr>
<td>VAS pain</td>
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</tbody>
</table>

VAS-Visual Analogue Scale
Effects of relaxation on skin barrier recovery

The average skin barrier recovery in the control group was 42.6% ($SD = 19.5\%$) compared with the relaxation pre-stripping group ($Mean = 54.2\%, SD = 22.1\%$) and the relaxation post-stripping group ($Mean = 52.2\%, SD = 25\%$). Several variables that have been associated with skin barrier recovery in other research were significantly correlated with skin barrier recovery in this study: age ($r = -0.29, p = .005$), number of strips ($r = -0.28, p = .007$), baseline TEWL ($r = -0.22, p = .037$), and amount TEWL elevated ($r = -0.55, p < .001$). These were therefore entered as covariates in Quade’s rank ANCOVA. Results showed that there was a significant overall difference between groups ($F(2,92) = 3.58, p = .032$, partial $\eta^2 = 0.074$). Post hoc comparisons using Fisher least significant difference tests revealed that there was a significant difference between the control group ($Mean rank = 27.72$) and the relaxation pre-stripping group ($Mean rank = 33.40, p = .047$), and a significant difference between the control group and the relaxation post-stripping group ($Mean rank = 31.71, p = .012$). Post hoc comparisons using Fisher least significant difference tests showed that there was no significant difference between the relaxation pre-stripping group and the relaxation post-stripping group ($p = .63$). This shows that the intervention, regardless of whether it was performed before or after tape stripping, improved skin barrier recovery compared with the control group when covariates were controlled for (Figure 4).

To test the model without covariates, we performed a Kruskal-Wallis $H$ test between the three groups, and the difference between groups became non-significant ($H = 4.04, p = .13$). However, in a Mann-Whitney test without covariates, there remained a significant difference between the control group and the relaxation groups when the relaxation groups were pooled together (median control = 39.89, median relaxation pooled = 48.36, $U = 688.00, z = -1.99, p = .047$, $r = -0.21$).
Figure 4. Percentage of skin barrier recovery measured 25 minutes after tape stripping between the control group, the relaxation pre-stripping group and the relaxation post-stripping group.

* = $p < .05$.

**Self-rated relaxation**

Figure 5a shows the changes in self-rated relaxation over four time points ($N = 121$). There was a significant main effect of time ($F(3,321) = 30.83, p < .001$). There was also a significant interaction effect between time and condition ($F(6,321) = 6.06, p = .003$). Pairwise comparisons were performed to investigate where the differences were over different time points and between groups. All groups relaxed from baseline to before tape stripping ($p < .001$). Before tape stripping, the relaxation pre-stripping group was more relaxed than the other two groups (relaxation pre-stripping compared with control, $p = .039$; relaxation pre-stripping compared with relaxation post-stripping, $p = .032$). The relaxation rating significantly increased from after tape stripping to follow-up in both intervention groups (relaxation pre-stripping, $p = .023$; relaxation post-stripping, $p < .001$), but not the control group. At follow-up, the relaxation post-stripping group differed significantly from the control group ($p = .012$), and the difference between the pre-stripping and post-stripping groups bordered on significance ($p = .080$).
Self-rated pain

Figure 5b shows the changes in self-rated pain over four time points. There was a significant main effect of time ($F(3,321) = 34.36, p < .001$), meaning that over time pain ratings significantly changed. There was no interaction effect. Pairwise comparisons show that in all three groups, pain significantly increased from before tape stripping to after tape stripping (control, $p < .001$; relaxation pre-stripping, $p < .001$; relaxation post-stripping, $p = .004$). This shows that pain did increase as a result of tape stripping. In all three groups, pain significantly decreased from after tape stripping to follow-up (control, $p = .005$; relaxation pre-stripping, $p < .001$; relaxation post-stripping, $p < .001$).

Figure 5b shows that the control group had higher levels of pain at follow-up. To further investigate this, both relaxation groups were pooled together to see if there were any differences between groups. Although there was no significant difference between groups after tape stripping, there was a significant difference between those groups that had performed relaxation and the control group at follow-up ($t(116) = -2.29, p = .042, r = 0.21$). An ANCOVA was conducted looking at the changes in pain over the session controlling for baseline levels of pain. By the follow-up point, those who performed relaxation had returned to baseline levels of pain ($Mean = -0.07, SD = 10.03$), while those in the control group had not ($Mean = 6.00, SD = 15.37$; $F(2,115) = 7.71, p = .006$, partial $\eta^2 = .06$).
Figure 5. a) Self-ratings of relaxation over the experimental session between the control group, the relaxation pre-stripping group and the relaxation post-stripping group; b) Self-ratings of pain over the experimental session between the control group, the relaxation pre-stripping group and the relaxation post-stripping group.
Discussion

This preliminary study showed that a relaxation intervention had a beneficial effect on skin barrier recovery regardless of whether the intervention was administered before or after wounding. Participants who completed the relaxation intervention had faster rates of skin barrier recovery (52–54% of skin barrier function recovered) after 25 minutes compared with the control group (43% of skin barrier function recovered).

The intervention worked to increase feelings of relaxation in comparison to quiet reading. Although we did not test the biological mechanisms involved, it would be reasonable to hypothesize that the mechanisms are similar for the two relaxation groups. Relaxation both before and after wounding may lower anxiety and autonomic arousal, reducing the presence of stress hormones that can affect the early stages of healing (Holden-Lund, 1988). Research using tape stripping (Robles, 2007) found that the greatest increase in skin barrier function occurs in the first 60 minutes after tape stripping, so the first 25 minutes is a critical stage.

Although previous research has shown that trait positive affect can buffer the effects of stress, leading to faster skin barrier recovery (Robles et al., 2009), this is the first study to show that a psychological intervention can improve skin barrier recovery. It adds to previous research showing that relaxation can improve collagen deposition in surgical wounds (Broadbent et al., 2012). In addition, the findings suggest that the intervention is effective whether it is delivered before or after wounding.

In contrast to other research (Garg et al., 2001), Perceived Stress Scale scores were not associated with skin barrier recovery. This may be because most of the sample were students and their stress levels may have varied considerably over the past 4 weeks as they completed examinations, tests, and assignments at various times. Measuring stress over the past 4 weeks may therefore have been too long and a shorter period may have been more appropriate.

This study found that there was a significant difference in ratings of pain over the session when comparing the two groups that performed relaxation with the control group. This supports
previous literature showing that relaxation post-surgery can have a beneficial effect on pain ratings (Gavito, Ledezma, Morales, Villaba & Ortega-Soto, 1999).

There are some limitations of this research. First, we found that there was some variation in TEWLmeter readings. This may be because the research was conducted in a ventilated room without a thermostat. Future research should use a climate-controlled room to avoid any potential problems. Miteva and colleagues (2006) found that compared with a climate controlled room, a ventilated room has more intra-instrumental fluctuations. This may account for why there were some inaccurate readings, which were subsequently excluded. Despite the number of participants excluded from the analysis, we found a medium effect size ($f = 0.28$). When computing a post hoc power analysis with our final sample size for skin barrier recovery (93 participants), we found that this yielded a power of 0.67. Second, the overall model testing the effects of relaxation pre-stripping and relaxation post-stripping on healing compared with the control condition was significant when including covariates. When excluding covariates, the model was only significant when the two relaxation groups were combined, which may reduce the robustness of the findings.

This study was performed in an experimental setting with a standardised method of producing a minor wound. This means that these findings cannot be directly applied to a clinical setting. However, the tape stripping model as a paradigm for healing may offer insight into how psychological interventions can have a beneficial effect and can help explore the impact of the timing of such interventions. Other research supports the current findings in clinical practice as relaxation has shown improvements in healing after surgery (Broadbent et al., 2012). This research is also pertinent to dermatology. Many skin conditions are aggravated by stress and research has found that interventions to help reduce stress have a positive impact. For example, research with patients with psoriasis has found that stress reduction interventions such as meditation and relaxation can help reduce flares associated with stress (Shenefelt, 2010). Hence, the tape stripping model offers a paradigm that could be applied to various areas of healing. However, more research
testing the timing of interventions in other wound types is needed before the results can be applied to clinical situations.

Another limitation is that the sample consisted mostly of healthy students and may not be an accurate representation of the general population. Also, no immunological measures were included in this research. Future research should focus on the immunological changes linked to the autonomic system that occur during relaxation in a wound healing paradigm. This may help understand in more detail how stress-reducing interventions during recovery affect healing. Lastly, the participants and researcher were not blind to group allocation (relaxation or control), which is potentially problematic. Although it is difficult to blind the participants, future studies should blind the researcher to group allocation.

Despite the limitations of this research, the findings are encouraging. The study is a good starting point for future research investigating the timing of interventions to improve healing. The study found that a simple relaxation intervention, either before or after tape stripping, improved skin barrier repair and decreased pain. If these findings can be replicated, this research may be clinically relevant, particularly for patients undergoing acute unplanned surgery by focusing on interventions during the recovery period.
Chapter 5: Role of sleep in skin permeability barrier recovery after disruption by tape stripping

Preface

Rationale

Chapter 4 described a randomised controlled trial investigating whether a brief relaxation intervention could improve skin barrier recovery. A subsidiary question investigated within this sample was whether sleep over the past 24 hours could influence skin barrier recovery. This was explored because not only has research in wound healing identified sleep as a variable that influences healing (Koschwanez et al., 2013) but recent work has highlighted the role of sleep in skin barrier recovery after tape stripping in women (Benham, 2015; Oyetakin-White et al., 2015).

The role of sleep in immunity and wound healing has been described in Chapter 2 and research indicates that lack of sleep can both be viewed as a stressor and can be caused by stress. People that have disrupted sleep often exhibit similar physiological changes to those under stress such as higher levels of cortisol, heart rate and oxygen consumption (Åkerstedt, 2006; Bonnet & Arand, 1996). In turn, people who are stressed have heightened physiological states, which mean they cannot switch off to sleep (Åkerstedt, Kecklund, & Axelsson, 2007).

It is important to understand the relationship between stress, sleep and skin function not only in terms of wound healing but also in relation to dermatological conditions. However, research indicates that sleep is rarely measured and that there is a lack of management guidelines for dealing with sleep issues in dermatological conditions (Thorburn & Riha, 2010). Research using tape stripping to impair skin function and measure skin barrier recovery can help delineate the relationship between sleep, stress and skin healing as a starting point for research in dermatological populations and wound healing, by showing the importance of sleep in immunological function.

The research presented here in Chapter 5 categorises participants as ‘good’ sleepers and ‘bad’ sleepers based on the amount of hours they reported sleeping over the past 24 hours to
investigate differences in skin barrier recovery. It was hypothesized that participants who reported sleeping longer would show faster skin barrier recovery compared to those who report sleeping for a shorter amount of time.

**Aims**

1. To investigate whether sleep over the past 24 hours impacts skin barrier recovery in a sample of healthy participants by comparing skin barrier recovery in ‘poor’ sleepers and ‘good’ sleepers based on a median split.
2. To build on previous research that has looked at skin barrier recovery in women only to replicate the results in a sample of men and women.
3. To identify the relationship between stress and sleep in this sample.

**Citation**

Abstract

Objectives: Previous research has shown poor sleep is associated with slower skin permeability barrier recovery after tape stripping in women. The aim of this research was to investigate this relationship in a sample of men and women.

Methods: One hundred and five participants rated their sleep over the past 24 hours and then underwent a standardised tape stripping procedure. Skin barrier recovery was calculated after 25 minutes by measuring transepidermal water loss. Participants were divided into two sleep groups based on the median of seven hours slept over the past 24 hours. Thirty eight participants who slept 7 and a half hours or more were classified as ‘good’ sleepers and 28 participants who slept for 6 and a half hours or less were classified as ‘poor’ sleepers. ‘Poor’ sleepers reported significantly higher stress levels than ‘good’ sleepers ($p = .02$).

Results: After controlling for perceived stress, ‘good’ sleepers had significantly faster skin barrier recovery ($Mean = 55\%, SD = 27\%$) compared to ‘poor’ sleepers ($Mean = 45\%, SD =26\%$; $F(1, 62) = 3.86, p = .05$, partial $\eta^2 = .06$. This effect was significant in both men and women.

Conclusions: The results support previous work showing poor sleep can impair skin barrier recovery in women and extend the findings to men. More research is needed to investigate the role of stress in the relationship between sleep and skin barrier function.
Introduction

Research suggests that sleep is critical to immune function and sleep deprivation can negatively influence the immune system (Lange, Perras, Fehm & Born, 2003; Ruiz, Andreson, Zager, Martins & Tufik, 2007). This is because sleep is integral for maintaining the interlinking immune-endocrine system, preserving homeostasis (Dickestein & Moldofsky, 1999). Sleep deprivation or even modest disturbances in sleep diminish immune functioning by altering cytokine profiles (Irwin, 2002) and elevating cortisol levels (Leproult et al., 1997). This has implications for wound healing as healing is a complex process, reliant on each aspect of the immune system functioning perfectly at each stage of healing to prevent prolonged inflammation and slow healing. Insomnia or changes to sleeping patterns activate the hypothalamic-pituitary-adrenal axis (Buckley & Schatzberg, 2005), releasing cortisol, which suppresses aspects of immune function involved in healing. Indeed research has found that higher levels of cortisol have been associated with slower healing after a punch biopsy wound (Ebrecht et al., 2003) and altered cytokine profiles have similarly been predictive of slower healing (Elias, Ansel, La Donna & Feingold, 1996). Cortisol is released in response to stress, and the link between stress and sleep means that higher stress may not only be a consequence of lack of sleep but also a contributing factor to sleep loss. Sleep is therefore important to investigate in relation to the immune response, cutaneous wound healing and interactions with stress.

Research looking at the impact of sleep on wound healing has found that experimentally created biopsy wounds heal faster if participants report at least 7 hours of sleep prior to wounding (Koschwanez et al., 2013). However other studies looking at sleep have failed to identify sleep as a variable effecting wound healing, possibly due to small sample sizes (Ebrecht et al., 2003).

There is evidence that lack of sleep can specifically impair the function of the epidermal barrier via the modulation HPA axis. As well as suppressing the immune system important to dermal healing, the release of glucocorticoids also affects skin tissue by hindering collagen synthesis and degradation (Kahan, Andersen, Tomimori & Tufik, 2009). Research looking at dermatological changes in skin permeability barrier recovery after wounding has found that sleep deprivation leads to slower
recovery. Specifically, research using the tape stripping model creating epidermal injury can provide insight into abnormal barrier function linked to cutaneous conditions and show the pattern of cytokine activation in the epidermis (Hirotsu, Rydlewski, Araújo, Tufik & Andersen, 2012). Research looking at psoriasis in an animal model shows mice that experience sleep deprivation have exacerbated symptoms caused by altered immune function in the epidermal barrier (Barrientos, Stojadinovic, Golinko, Brem & Tomic‐Canic, 2008). Research with humans has similarly found alterations in epidermal barrier function due to decreased sleep. In one study (Altemus et al., 2001), women were subjected to psychological stress (a job interview) or sleep deprivation. The results found that both psychological stress and sleep deprivation resulted in delayed skin permeability recovery 3 hours later, indicating the alteration of the HPA response in healing. Furthermore, recent research indicates that even reduced sleep can influence skin permeability barrier recovery. Sixty healthy women categorised as ‘good’ sleepers (defined using the Pittsburg Sleep Quality Index (PSQI) by scores less than 5, and slept 7–9 hours a night) were compared to poor sleepers (PSQI scores greater than 5, and slept less than 5 hours a night). The results found that skin barrier recovery of the optimal sleepers was 30% faster than poor sleepers 72 hours after tape stripping (Oyetakin-White et al., 2015). Similarly, better self-reported sleep assessed by asking participants how long they slept on average per night was associated with faster skin barrier recovery amongst 29 healthy female students half an hour later and 3 hours later (Benham, 2015). A limitation of these skin barrier recovery studies is that they have only been conducted with women.

The aim of this study was to determine whether sleep had an influence on skin permeability barrier recovery within the first 30 minutes of tape stripping in both men and women. This time point was chosen because only one previous study has looked at this short time frame, and yet it has been shown to be a critical time period due to the formation of intercellular lipid bilayer structures (Denda, Sokabe, Fukumi-Tominaga & Tominaga, 2007), although the mechanism through which this occurs is not known.
The objectives of this study was to look at differences in self-reported sleep before participants underwent a tape stripping procedure and whether this influenced skin barrier permeability recovery 25 minutes after wounding. It was hypothesized that participants reporting poor quality sleep would have slower rates of recovery compared to participants reporting good quality sleep, similar to previous studies, and the results would hold for both men and women.

**Methods**

**Sample**

One hundred and twenty-one participants were recruited from the local community and university campus through flyers, email and online advertisements as part of a larger study focusing on the relationship between relaxation and skin permeability barrier recovery (Robinson et al., 2015). For this paper, allocation to a relaxation group was controlled for in the analysis. Participants had to be over the age of 16 years and provide written informed consent. Participants were excluded if they had allergies to tape or adhesives, an inflammatory dermatological condition affecting the forearm or were taking immunomodulatory medication. Ethics approval was obtained from The University of Auckland Human Participants Ethics Committee (UAHPEC; Reference number 010545). Sixteen people were excluded from the sample due to unreliable or incomplete skin barrier recovery data, which left 104 participants with valid data (Figure 6).

**Procedure**

Participants took part in a 90 minute session at The University of Auckland Clinical Research Centre in a ventilated room, whereby the temperature was controlled by the central university heating system (average temperature 23°C, average humidity 56%). These conditions were consistent with the guidelines for operating the transepidermal water loss meter (TEWLmeter) probe recommended by the manufacturer. Participants were asked not to drink caffeinated drinks for one hour before the session or shower or exercise for half an hour before the session. Participants were also instructed not to apply moisturizer to their arms on the day of the session. At
the beginning of the session participants were asked to complete a baseline questionnaire relating to sleep, stress and pain. Participants were randomised to take part in a 20 minute private relaxation session or to read quietly in accordance with the larger study outcome. After this period baseline measures of skin barrier permeability were taken from four areas on the inner arm. Skin barrier disruption was performed using a standardised tape stripping procedure for all participants. After tape stripping, skin barrier permeability was measured again and participants completed another brief questionnaire on perceived pain and stress. They were then told that they need to wait 20 minutes to see how their skin recovered. Participants were asked to complete a final questionnaire in pain and then a follow-up measure of skin barrier permeability was taken. All participants were given a $10 voucher as compensation for their time. Participants were only required to come to the clinic for one appointment to reduce participant burden. There were no other follow-up sessions.

*Tape stripping and skin barrier recovery*

Tape stripping is a procedure that has been repeatedly show to disrupt skin barrier permeability and is common in dermatological research (Fluhr et al., 2006). The tape stripping method used in this study followed protocol from previous research looking at skin permeability barrier recovery (Garg et al., 2001; Robles, 2007). Baseline skin barrier function was measured by obtaining baseline readings of TEWL using TEWLmeter (Courage Khazaka, Germany), as used in previous research (Benham, 2015), which measures evaporation of water from the skin (evaporation rate in g/h/m²). TEWL indicates the skin’s ability to prevent water loss from the epidermis. Three 1cm² test areas were marked adjacently down the inside of the forearm, starting 1cm below the elbow crease. Additionally, one extra area was marked, a centimetre apart from the test area. This area was a control site and was not subjected to tape stripping. A baseline measure of all four sites was taken using the TEWLmeter, where the probe was placed on the skin for one minute per site with a reading obtained each second. Higher TEWL indicates that more water loss is occurring through the skin and skin barrier permeability is impaired. Researchers were blinded to the sleep status of each participant during tape stripping and TEWL measurement.
The three test sites were then dry shaved with a disposable razor to ensure that no hair was pulled out during tape stripping. Standard tape (Scotch Clear Packaging Tape, 3M) was applied 20 times to the three test areas to obtain at least a 15 g/h/m² increase from baseline. After the initial 20 strips of tape the first area was tested to see if TEWL had been elevated by 15 g/h/m². If more strips of tape were required the area was tested at regular intervals to see if the TEWL was elevated. Tape stripping stopped after a maximum of 40 strips of tape had been applied. A measurement for each site including the control site was taken to determine the elevated level. For the rest of the session participants kept their arm uncovered and were instructed not to touch the area. At the end of the session the skin was measured again using the TEWLMeter. The 25 minute time period was chosen as previous studies indicate that the greatest increase in healing occurs within the first hour (Benham, 2015; Robles, 2007). There were no other follow-up points to reduce burden to participants.

**Transepidermal water loss analysis**

Twenty consecutive measurements with a standard deviation below 0.5 were averaged to give an overall TEWL reading for each site at baseline, after tape stripping and after 25 minutes recovery. Skin barrier recovery was computed as a percentage based on the formula used in several studies (Garg et al., 2001):

\[
\text{Recovery} = \frac{(\text{TEWL}_{\text{post tape stripping}} - \text{TEWL}_{25 \text{ min follow-up}})}{(\text{TEWL}_{\text{post tape stripping}} - \text{TEWL}_{\text{baseline}})} \times 100\%
\]

Greater values reflect greater skin permeability barrier recovery. Three areas of the skin were subjected to tape stripping to ensure accurate data. One hundred and 19 participants had complete readings at all three time points. Data for each individual site was excluded if recovery over 200% (similar to previous research, (Robles, 2007); three participants excluded), the baseline reading was too high (2 SDs above the mean, two participants excluded), skin was not sufficiently impaired (less than 5 g/h/m², nine participants excluded) or participants scratched the tape stripped area during recovery resulting in no recovery (one participant excluded). The remaining sites were averaged similar to previous research (Robles, 2007). If the three readings were not within 30 g/h/m² of each
other the two closest in value of the three sites were averaged. After the exclusions of these sites 104 participants had at least one reliable reading.

**Demographic and psychological measures**

Participants were asked about their age, gender and ethnicity. Participants were asked to report how many hours sleep they had over the past 24 hours taken from the Pittsburg Sleep Quality Index (PSQI; Buysse, Reynolds, Monk, Berman & Kupfer, 1989). Based on this question, participants were rated as ‘good’ sleepers or ‘poor’ sleepers using a median split. Previous studies that have looked at the role of sleep in skin barrier recovery have similarly categorized participants as ‘good’ sleepers and ‘poor’ sleepers based on the single question from the PSQI assessing hours sleep each night (Benham, 2015).

Participants were asked to complete the Perceived Stress Scale (PSS; Cohen & Willimson, 1988) at baseline. This 10 item scale was used to determine how much participants felt their lives were unpredictable, uncontrollable and stressful. Respondents were asked to indicate how often they felt a certain way over the last month on a scale from 0 (“never”) to 4 (“very often”). Higher scores indicate higher perceived stress.

Participants were also asked to complete a visual analogue scale assessing pain at baseline. Participants were asked to mark on a 100 mm line how much pain they were currently feeling. The anchors were “no sensation of pain” and “most sensation of pain imaginable”.

All data were analysed using the Statistics software package ‘IBM SPSS 19.0 for Windows’. Spearman correlations were conducted to determine the relationship between sleep and skin permeability recovery due to non-normal data distributions. T-tests and chi square analysis and equivalent non-parametric test were conducted to determine if there were any differences between groups at baseline.

For the main outcome (skin barrier recovery), ANCOVAs controlling for baseline stress and relaxation allocation group were conducted between ‘good’ sleepers and ‘poor’ sleepers in men and women together, and then in men and women separately. Perceived stress was entered as a
covariate in the ANCOVAs because stress has been shown to be related to slower skin barrier recovery in previous work (Altemus et al., 2001). In addition, we controlled for relaxation condition because our study showed relaxation improved skin barrier recovery (Robinson et al., 2015).

**Results**

The median amount of sleep reported over the past 24 hours in the sample of 104 participants was 7 hours. Using this median, ‘good’ sleepers were defined as those that slept for 7 and a half hours or more ($N = 38$) and ‘poor’ sleepers were defined as those that slept for 6 and a half hours or less ($N = 28$). Those reporting 7 hours sleep were excluded from the analysis ($N = 38$) to create clear groups (Figure 6).

Of these 66 participants classified as ‘good’ or ‘poor’ sleepers, 49 participants were female (74%). The mean age was 24.06 years ($SD = 10.21$; $Mean BMI = 23.04$, $SD = 3.82$). The sample consisted of mostly Europeans (59%), while the rest of the sample were Asian (27%) or identified as ‘Other’ (14%). Table 4 shows there were no differences between ‘good’ and ‘poor’ sleepers on demographic variables. ‘Good’ sleepers reported an average of 8.21 hours sleep over the past 24 hours ($SD = 0.65$), whereas ‘poor’ sleepers reported an average of 5.65 hours sleep over the past 24 hours ($SD = 0.82$).

There were no significant differences in self-reported pain between ‘good’ and ‘poor’ sleepers (Table 4). However, there was a significant difference between groups on perceived stress, $t(64) = 1.36$, $p = .02$, $r = .28$, whereby ‘good’ sleepers reported lower levels of stress than ‘poor’ sleepers (see Table 4).
Responded to study advertisement \((N = 142)\)

Excluded \((n = 21)\)
- Decided not to participate \((N = 12)\)
- Could not commit to session \((N = 5)\)
- Excluded due to skin condition \((N = 3)\)

Completed study \((N = 121)\)

Analysed for skin barrier recovery \((N = 104)\)

Excluded \((N = 17)\)
- Incomplete data \((N = 2)\)
- Recovery over 200% \((N = 3)\)
- Baseline measures were above 30 g/h/m\(^2\) \((N = 2)\)
- Skin not elevated or elevated by less than 5 g/h/m\(^2\) \((N = 9)\)
- Interference with the site \((N = 1)\)

Excluded from analysis
- Slept a median of 7 hours \((N = 38)\)

Classified as ‘good’ sleepers (slept for 7.5 hours or more; \(N = 38\))
- Females \((N = 30)\)
- Males \((N = 8)\)

Classified as ‘poor’ sleepers (slept for 6.5 hours or less; \(N = 28\))
- Females \((N = 19)\)
- Males \((N = 9)\)

Figure 6. CONSORT flow diagram.
Table 4. Demographic and baseline characteristics of 'poor sleepers' and 'good sleepers' categorized using a median split.

<table>
<thead>
<tr>
<th></th>
<th>Poor sleepers (N = 28)</th>
<th>Good sleepers (N = 38)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (Mean, SD)</td>
<td>25.32 (13.04)</td>
<td>23.58 (10.57)</td>
<td>.772</td>
</tr>
<tr>
<td>Gender (N female, %)</td>
<td>19 (68%)</td>
<td>30 (79%)</td>
<td>.313</td>
</tr>
<tr>
<td>Ethnicity (N, %)</td>
<td></td>
<td></td>
<td>.207</td>
</tr>
<tr>
<td>European</td>
<td>16 (57%)</td>
<td>23 (61%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>8 (29%)</td>
<td>10 (26%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4 (14%)</td>
<td>5 (13%)</td>
<td></td>
</tr>
<tr>
<td><strong>Skin permeability barrier</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of strips required to elevate skin barrier</td>
<td>22.86 (5.34)</td>
<td>24.73 (6.87)</td>
<td>.278</td>
</tr>
<tr>
<td>Baseline TEWL (Mean, SD)</td>
<td>14.80 (3.49)</td>
<td>14.10 (4.31)</td>
<td>.204</td>
</tr>
<tr>
<td>Amount TEWL elevated by (Mean, SD)</td>
<td>27.37 (22.22)</td>
<td>22.91 (19.47)</td>
<td>.295</td>
</tr>
<tr>
<td><strong>Psychological variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perceived Stress Scale (PSS; (Mean, SD)</td>
<td>22.25 (3.50)</td>
<td>20.16 (3.74)</td>
<td>.022</td>
</tr>
<tr>
<td>Self-reported pain (VAS; (Mean, SD)</td>
<td>14.66 (19.96)</td>
<td>9.41 (12.71)</td>
<td>.307</td>
</tr>
</tbody>
</table>
Recovery after tape stripping

In the ANCOVA with the combined gender sample, the covariate stress was a significant predictor of skin permeability barrier recovery, $F(1, 62) = .4.13, p = .048$, $\eta^2_{p} = .06$. The covariate relaxation condition was not significant, $F(1, 26) = .55, p = .462$, $\eta^2_{p} = .01$. After controlling for these covariates, sleep was significant $F(1, 62) = 3.86, p = .047$, $\eta^2_{p} = .06$. Skin permeability barrier recovery was significantly better in ‘good’ sleepers (Adjusted mean = 57%, SE = 4.31) compared to ‘poor’ sleepers (Adjusted mean = 43%, SE = 5.05; Figure 7). When stress was not entered as a covariate there was no longer a significant difference in skin barrier recovery between ‘good’ and ‘poor’ sleepers, $F(1, 63) = 2.04, p = .163$, $\eta^2_{p} = .03$.

When the ANCOVA was conducted with men only, similar results were obtained. The covariate perceived stress was significant, $F(1, 13) = 11.60, p < .001$, $\eta^2_{p} = .47$. The covariate relaxation condition was not significant $F(1, 13) = .54, p = .484$, $\eta^2_{p} = .04$. After controlling for these covariates, there was a significant differences in skin barrier recovery between ‘good’ and ‘poor’ sleepers; $F(1, 13) = 5.04, p = .042$, $\eta^2_{p} = .28$ (Figure 7). When stress was not entered as a covariate there was no longer a significant difference in skin barrier recovery between ‘good’ and ‘poor’ sleepers, $F(1, 14) = .00, p = .955$, $\eta^2_{p} = .00$.

The ANCOVA conducted with women only showed that neither stress $F(1, 45) = .77, p = .394$, $\eta^2_{p} = .02$ nor relaxation condition were significant predictors of skin barrier recovery $F(1, 45) = .16, p = .697$, $\eta^2_{p} = .00$. After controlling for these covariates, there were significant differences in skin barrier recovery between ‘good’ and ‘poor’ sleepers, $F(1, 45) = 4.28, p = .045$, $\eta^2_{p} = .09$ (Figure 7). When perceived stress scores were not entered as a covariate there was still a significant difference in skin barrier recovery between ‘good’ and ‘poor’ sleepers, $F(1, 46) = 3.82, p = .046$, $\eta^2_{p} = .08$. 
Figure 7. Adjusted means showing percentage of skin permeability recovery 25 minutes after tape stripping in good and poor sleepers in the both men and women, men only and women only after controlling for relaxation allocation and stress.

*p < .05
Discussion

This research indicates that sleep and stress are critical variables in skin barrier permeability repair. After controlling for perceived stress, participants who reported having more sleep over the 24 hours prior to tape stripping had significantly better skin barrier permeability recovery than those who reported having less sleep. While this effect has been shown previously in women (Altemus et al., 2001; Benham, 2015; Oyetakin-White et al., 2015), this is the first study to demonstrate the effect in men as well as women.

Similar to previous research (Altemus et al., 2001), this study found that people who reported higher perceived stress reported less sleep. In women, poor sleep contributed to worse skin barrier repair even when stress was not controlled for statistically in the analysis. However, in men the difference in skin barrier permeability repair between good and poor sleepers was only significant when stress was controlled for in the analysis. This may suggest that the relationship between stress, sleep, and skin function, could differ by gender, although the small sample of men means these results need to be replicated before this conclusion can be made. Stress may impair sleep, and both may affect skin barrier permeability repair.

With regards to gender, previous work has shown that in females but not males, less sleep is associated with higher inflammatory biomarkers (Miller et al., 2009). It has been proposed that higher levels of testosterone are associated with lower levels of IL-6 and therefore may blunt the negative effects of poor sleep (Yaggi, Araújo & McKinlay, 2006). A similar mechanism may operate in which testosterone has protective effects on skin barrier recovery in males with poor sleep. However, more research is needed to investigate this.

Different time points can be used to assess skin barrier recovery. Oyetakin-White and colleagues (2015) assessed skin barrier recovery 1, 24 and 72 hours after tape stripping, finding a significant difference between groups after 72 hours only. Our research investigated the immediate recovery period, and our results were very similar to Benham (2015) who found that self-reported sleep was associated with skin barrier recovery at both 30 minutes and 3 hours after tape stripping.
Furthermore, the speed of skin permeability barrier recovery after 30 minutes in Benham (2015), are close to the findings reported in this paper, possibly because the equipment used in both studies was the same, whereas other research has used a TEWL meter manufactured by different companies.

There are some limitations to this research. Firstly, there were fewer men than women in this study, limiting the power of the study to detect effects in men. More research needs to be conducted looking at gender differences in the impact of reduced sleep on healing, as well as the role of stress. Along with the work by Benham (2015), we used simple self-reported measures of sleep which may not be optimal. Other research looking at the effect of sleep on immune system modulation indicates that individuals tend to overestimate sleep duration and underestimate how much time they are awake at night (Lauderdale, Knutson, Yan, Liu & Rathouz, 2008) and that measuring sleep with wrist actigraphy is more accurate (Prather, Janicki-Deverts, Hall & Cohen, 2015). Another consideration is that people who have different sleep patterns may have differences in circadian rhythms, which can influence skin surface temperature and pH (Ghadially et al., 1995).

The differences in TEWL could be a result of these differences and this should be explored in future work.

In order to further investigate the mechanisms underlying the relationship between sleep, skin permeability barrier recovery, and stress, future studies should include cortisol. As this study was a preliminary investigation, biological indices such as cortisol were not assessed but may be a modulating factor underlying immunological changes. Previous research has indicated that lack of sleep elevates cortisol levels (Leproult et al., 1997) and cortisol levels are known to impair skin barrier recovery when elevated due to a laboratory induced stressor (Garg et al., 2001) as well as aggravate various cutaneous dermatoses associated with abnormal skin barrier function (e.g., psoriasis and dermatitis; Tausk & Nousari, 2001). Furthermore, animal models have found that glucocorticoids impair skin integrity by inhibiting the synthesis of lipids, preventing the production of
lamellar membranes in the stratum corneum of the epidermis (Kahan, Andersen, Tomitori & Tufik, 2010).

In conclusion, these results support the previously observed association between sleep disturbance and impaired skin permeability barrier recovery in women, and extend the findings to men. This research shows that even moderate differences in sleep duration can influence skin barrier function, which is important to note given research showing a trend in decreasing hours of sleep in modern society (Bixler, 2009). The findings may be clinically relevant for people with dermatological conditions, and highlight the importance of considering sleep in the management of skin disease.
Chapter 6: The effects of expressive writing before or after punch biopsy on wound healing

Preface

Rationale

Chapter 3 identified that expressive writing interventions have been used to help facilitate wound healing in experimental settings. The two studies to date that have been conducted assessing wound healing prior to a biopsy wound both found an expressive writing intervention to benefit wound healing (Koschwanez et al., 2013; Weinman et al., 2008). These studies both employed similar methodologies, and implemented the expressive writing intervention approximately two weeks prior to receiving a standardised punch biopsy wound.

In this thesis, Study 1 investigated the timing of a relaxation intervention on skin barrier recovery and found that regardless of when the intervention was performed (either directly before or directly after tape stripping) it had beneficial effects on skin barrier recovery. Unlike relaxation, which appears to have a direct stress-buffering effect and reduces sympathetic activity, expressive writing may have more of a gradual effect on stress and initially may increase distress (Pennebaker, Hughes & O’Heeron, 1987). It is thought that expressive writing helps acknowledge distressing events, create a narrative and reduce physiological inhibition (Pennebaker, 1985), but the outcomes of this process take time because the cognitive processing does not occur instantly. If the intervention is administered following wounding it may have a different effect on healing, compared to if it is administered prior to wounding. Research with expressive writing in clinical populations, shows that benefits in illness outcomes are evident after different periods of time (Smyth, Stone, Hurewitz & Kaell, 1999). Hence the timing of such an intervention may be critical for wound healing, particularly at certain points in the inflammation process, which may dictate subsequent improved or delayed healing (Godbout & Glaser, 2006).
Given that expressive writing in previous wound healing research (Koschwanez et al., 2013; Weinman, et al., 2008) is administered prior to wounding, the second experimental study presented in this thesis in Chapter 6 (Study 2) sought to explore whether timing of an expressive writing intervention was influential on wound healing. Study 2 was a randomised controlled trial conducted with a healthy population, designed to assess whether an expressive writing intervention conducted prior to or after receiving a punch biopsy wound could impact wound healing. Again this allowed for the timing of psychological interventions to be explored and will provide more insight as to when interventions are most appropriate to implement. It was hypothesized that participants randomised to perform expressive writing prior to wounding would have faster healing than a control group who wrote about a neutral topic during this period. Furthermore, those that performed expressive writing after wounding would also have better recovery in comparison to a control group who wrote about a neutral topic after wounding.

**Aims**

1. To investigate whether an expressive writing intervention could improve wound healing after a punch biopsy compared to a control group who wrote about a neutral topic.

2. To explore whether the timing of the expressive writing intervention (either before or after wounding) influenced wound healing.

3. To identify whether changes in stress and mood occurred as a result of expressive writing.

**Citation**

Abstract

Objectives: Recent studies have shown that written emotional disclosure (expressive writing) performed two weeks prior to wounding improves healing of punch biopsy wounds. In many clinical settings, it would be more practical for patients to perform this intervention after wounding. The aim of this study was to investigate whether expressive writing could speed the healing of punch biopsy wounds if writing was performed after wounds were made.

Methods: One hundred and twenty-two healthy participants aged between 18 and 55 years were randomly allocated to one of four groups in a 2 (intervention) by 2 (timing) design. Participants performed either expressive writing or neutral writing, either before or after receiving a 4mm punch biopsy wound. Wounds were photographed on day 10 (primary endpoint) and day 14 after the biopsy to measure epithelialisation. Participants also completed questionnaires on stress and affect two weeks prior to the biopsy, on the day of biopsy and two weeks after biopsy.

Results: There was a significant difference in healing at day 10 between groups, $\chi^2(3, N = 97) = 8.84$, $p = .032$. A significantly greater proportion of participants who performed expressive writing before the biopsy had fully re-epithelialised wounds on day 10 compared to participants who performed neutral writing either before or after wounding, with no other significant differences between groups. Amongst people who wrote expressively after wounding, those who finished writing over the first 6 days were significantly more likely to be healed at 14 days than those who finished writing later. There were significant differences in positive and negative affect over the healing period between the pre and post expressive writing groups.

Conclusions: Expressive writing can improve healing if it is performed prior to wounding. Performing expressive writing after wounding may be able to improve healing depending on the timing of writing and wound assessment. Expressive writing causes affect to worsen followed by a subsequent improvement in affect and it is important to consider this in the timing of intervention delivery. Further research with patient groups is required to determine the clinical relevance of these findings.
Introduction

Psychological stress can negatively influence wound healing in both healthy and clinical populations (Walburn et al., 2009). Stress has been shown to impair the healing of small punch biopsy and blister wounds, as well as slow recovery of skin barrier function after experimental wounds have been created (for example, Altemus et al., 2001; Kiecolt-Glaser et al., 1995; Kiecolt-Glaser et al., 2005; Marucha et al., 1998; Muizzuddin et al., 2003). In addition, stress has been observed to impair the healing of surgical and chronic wounds (Broadbent et al., 2003; Cole-King & Harding, 2001; Maple et al., 2015).

There is initial evidence that psychological interventions can improve wound healing. Written emotional disclosure (also known as expressive writing) involves writing emotionally about past traumatic events. It is thought that writing about stressful or traumatic events and upsetting emotions can help a person process the event, which in turn can decrease stress and rumination (Pennebaker, 1997). Expressive writing has been shown to have beneficial effects on the healing of punch biopsy wounds compared to writing factually about a neutral topic (Weinman et al., 2008; Koschwanez et al., 2013). Consistent with these studies, a systematic review showed that emotional disclosure has significant effects on immune parameters (Frattaroli, 2006). Researchers have suggested that expressive writing may be useful for patients who have clinical wounds (Weinman et al., 2008).

An important consideration in the translation of this laboratory-based research to clinical populations is the timing of intervention delivery. The previous two studies on emotional disclosure and wound healing both administered the intervention two weeks prior to wounding with instructions to write over the following three consecutive days (Weinman et al., 2008; Koschwanez et al., 2013). However, administering a writing intervention prior to surgery may be impractical in some situations, such as when emergency surgery is performed, when the date of surgery is scheduled at late notice or surgery is postponed. In the case of chronic wounds, the wound would already have been present for a considerable amount of time. In these situations, it would be more
practical for patients to perform expressive writing after the wound has occurred. It is therefore important to investigate whether expressive writing can improve healing when performed after wounding. Previous research indicates that other mind body interventions, such as hypnosis, when performed after surgery can have positive effects on wound healing (Ginandes et al., 2003).

To date only one study has explored whether altering the timing of a psychological intervention has differential effects on wound healing (Robinson et al., 2015). During this exploratory study participants underwent a tape stripping procedure, designed to damage the skin on the forearm. Participants were randomized to one of three conditions: relaxation for 20 minutes immediately prior to skin damage, relaxation for 20 minutes immediately after skin damage or no relaxation. Participants in the two relaxation conditions had faster skin barrier recovery than the no-relaxation group regardless of whether they did the intervention before or after the tape stripping procedure. This suggests that relaxation can have beneficial effects on healing even if performed after wounding. However, relaxation has immediate effects on physiology (Jacobs, 2001), whereas the beneficial effects of expressive writing may take longer to occur (Pennebaker, 1993). Expressive writing causes a short term increase in distress, negative mood and physical symptoms (Booth et al., 1997). However, often participants report they feel better and they have physiological improvements in the long term after expressing their problems rather than keeping them bottled up (Murray & Segal, 1994). For example, immediately after expressive writing, patients with rheumatoid arthritis had poorer functioning than a control group, but had significantly better functioning after 3 months. The authors attribute this to the time it takes to process negative events (Kelley, Lumley & Leisen, 1997).

The aim of this study was to compare the effectiveness of an expressive writing intervention performed either pre or post wounding on the healing of punch biopsy wounds. Previous research found that expressive writing performed prior to wounding significantly improved healing assessed ten to twelve days after the biopsy, and 90% of all wounds were healed by day 14 (Koschwanez et al., 2013). Therefore, the primary endpoint for this study was whether wounds were healed or not at
ten days post-biopsy. Wound healing was also assessed at day 14, although it was expected that the majority of wounds would be healed by this time point. It was hypothesized that the expressive writing intervention would improve healing when performed either before or after wounding compared to the control groups performing neutral writing. A secondary aim was to explore how stress and mood differed over the healing period between the groups who performed expressive writing pre or post wounding. It was hypothesised that both intervention groups would experience increased negative affect while writing, and improvements in affect would occur after writing had finished.

Methods

Sample recruitment

Participants were recruited from the local community and university campus through flyers, email and online advertisements. Participants had to be aged between 18 and 55 years and able to give written informed consent. Participants were excluded if they were pregnant, had allergies to local anaesthetic, smoked, had any inflammatory skin diseases, chronic illnesses, immunological-related health problems or were taking medication that affects immune functioning (such as antibiotics or corticosteroids). Ethics approval was obtained from The University of Auckland Human Participants Ethics Committee (UAHPEC). Previous research found the effect of expressive writing on wound healing to be moderate, Cramer’s V = .35 (Koschwanez et al., 2013). With power set at .80 and a two tailed significance level of α = .05, the total sample size required for an ANCOVA analysis with four groups was calculated using G*Power to be 120 (30 participants per group).

Procedure

Two weeks before the first scheduled appointment, participants were contacted by phone or email and asked to complete a baseline questionnaire online to gather information about demographics, general health, sleep, stress levels, and affect. Participants were then randomized to one of four groups: expressive writing task pre biopsy, control writing task pre biopsy, expressive writing task post biopsy and control writing task post biopsy. Randomization was performed by a
person uninvolved in this project using a random number generator. After the participant completed the baseline questionnaire, the researcher opened a sealed opaque envelope revealing which group the participant was allocated to. Those allocated to write pre biopsy were then given the intervention instructions online and asked to complete the writing task over the next three days prior to their clinical appointment 14 days later. Those allocated to write after the biopsy were simply asked to come back in 14 days for the biopsy appointment.

During this appointment a dermatologist performed a 4mm punch biopsy to the upper inner arm 7cm proximal to the medial epicondial of the humerus (inner upper arm). The area was cleaned with an alcohol solution and the tissue was anaesthetized using 1% lignocaine and 1:200,000 adrenaline. Once the wound stopped bleeding it was photographed using an EOS 100D Canon camera (Canon Ltd., Tokyo, Japan) with a Canon Ultrasonic EF 100-mm f/2.8 Macro USM lens and Canon ringflash. In order to calibrate each photograph, the wound was photographed with a standard-sized adhesive dot (1/4-in. diameter; Avery Dennison, Brea, CA). After photography, the dot was removed, and the wound was sealed with DuoDERM Extra Thin hydrocolloid dressing (ConvaTec, Skillman, NJ) and a water-proof plaster (Cutifilm Plus; Smith & Nephew, London, UK). Hydrocolloid dressings have previously been used in wound healing studies to provide a moist wound healing environment (Kiecolt-Glaser et al., 1995, Korting, Schoellmann & White, 2011). This means that there is faster epithelialisation compared with uncovered wounds and the prevention of eschar formation allows for more accurate assessments of wound reepithelialisation. Participants were instructed to keep the underlying dressing on until the next appointment, when the researcher would clean and redress the wound. After the biopsy all participants were asked to complete a second online questionnaire within 24 hours. Those allocated to the post biopsy writing groups were also asked to complete the online writing task over the next three days.

Ten days after the biopsy, all participants were scheduled for a follow-up appointment during which the wound area was gently cleaned with sterile saline and gauze, photographed, and redressed with DuoDERM Extra Thin and a water-proof plaster. Fourteen days after the initial biopsy
participants were scheduled to have a final appointment, during which the wound was photographed for the last time. Participants were given a $40 voucher as compensation for their time and were asked to complete a final questionnaire online.

**Expressive writing intervention**

The writing intervention was based on a standardized script used in previous studies (Koschwanez et al., 2013; Pennebaker, 1993; Weinman et al., 2008). Participants in the emotional disclosure groups were asked to write about their “deepest thoughts and feelings about a traumatic, upsetting experience of your entire life.” If they did not have a traumatic experience they were instructed to write about a significant life-changing event. Ideally participants were asked to write about something they had not discussed in great detail with anybody else.

Participants in the control groups were asked to write about how they spent their time. For each session they were first asked to write about the past week, then the past 24 hours and finally their plans for the upcoming week. They were specifically instructed to write about the facts, omitting any emotions.

Participants were asked to start writing the next day and to write for 3 consecutive days at home, for 20 minutes a day, without concern for spelling or grammar. Participants were reminded daily by email or text to complete the task each day. They were told that it was alright if they missed a day, but to make sure they wrote the next day instead. Participants used a secure online portal to complete their writing tasks. To ensure anonymity participants used an individual code to log on to the portal and at the end submitted their writing for analysis. Participants were told that their writing would not be read, but analysed by the computer programme (Linguistic Inquiry and Word Count; Pennebaker, Booth & Francis, 2007). This programme automatically categorizes digitized text into multiple psychologically relevant categories (Pennebaker & Francis, 1996). Previous research has found that expressive writing submitted online is effective (Sheese, Brown & Graziano, 2004). After each writing session, participants were asked to report how much emotion they revealed,
ranging from 1 ("not at all") to 5 ("a great deal") (Pennebaker, Colder & Sharp, 1990), as a manipulation check.

**Measures**

Participants completed questionnaires at three time points: two weeks prior to the biopsy (baseline), immediately after the biopsy, and 14 days after the biopsy (follow-up). The baseline questionnaire included questions about demographics, health behaviours, sleep, stress and affect. The questionnaires given immediately after the biopsy and at follow-up only included questions on sleep, stress and affect.

**Demographic and psychological measures**

Participants were asked their age, weight, height, ethnicity and education level. Health behaviour data were collected regarding alcohol consumption, smoking status, exercise regularity, and diet. Alcohol consumption was rated over the past 3 months from 1 (never) to 6 (everyday). On days participants did drink they were asked to rate how many drinks they had ranging from 1 (0 drinks) to 7 (12 or more drinks). Participants were asked to rate how often they did physical activity for 30 minutes over an average week from 1 (never) to 8 (everyday). They were asked to rate their diet over the past week from 1 (very poor) to 5 (very good).

Sleep was assessed using the Pittsburgh Sleep Quality Index (PSQI; Buysse et al., 1989). The scale consists of 19 questions used to generate seven component scores and an overall global score. The items asked participants to report the time they go to sleep, the time they wake and the amount of sleep they get. They are also asked to rate on a scale from 0 (not at all) to 3 (3 or more times a week) any sleep disturbance over the last month due to commonly experienced problems that disrupt sleep. The scale demonstrated good internal reliability (Cronbach’s α = .79)

The 10 item Perceived Stress Scale (PSS; Cohen & Williamson, 1988) was used to determine how much participants felt their lives were unpredictable, uncontrollable and stressful. Respondents were asked to indicate how often they felt a certain way over the last month on a scale from 0 (never) to 4 (very often). This scale has been found to be reliable and valid previously in student
populations (Roberti, Harrington & Storch, 2006). In this study, the scale had good internal reliability (Cronbach’s α = .85).

Affect was assessed using a modified version of the actual affect subscale of the Affect Valuation Index (AVI; Tsai, Knutson & Fung, 2006). The scale consisted of 25 items designed to measure how much participants were experiencing certain types of affect on a 5-point Likert scale ranging from “not at all” to extremely”. In the original wording, participants were asked how they felt over the course of typical week, whereas in this study participants were asked how they felt in the current moment. These modified instructions have been used in previous research and shown to be sensitive to changes in affect over time (Nair, Sagar, Sollers, Consedine & Broadbent, 2015). The scale is comprised of eight factors with three or four items in each factor: high arousal positive affect (HAP; strong, excited, enthusiastic), low arousal positive affect (LAP; calm, relaxed, rested, peaceful), positive affect (PA; happy, content, satisfied), negative affect (NA; sad, lonely, unhappy), high arousal negative affect (HAN; hostile, fearful, nervous), low arousal negative affect (LAN; dull, sleepy, sluggish), low arousal affect (LA; quiet, still, passive) and high arousal affect (HA; aroused, surprised, astonished). This scale has been found to be reliable and valid in different cultures (Tsai et al., 2006) and in this study had good internal reliability for each subscale (HAP Cronbach’s α = .80; PA Cronbach’s α = .81; LAP Cronbach’s α = .86; LA Cronbach’s α = .72; LAN Cronbach’s α = .81; NA Cronbach’s α = .78; HAN Cronbach’s α = .71; HA Cronbach’s α = .82).

Wound healing assessment

The primary outcome for the trial was wound reepithelialisation at 10 days post-wounding. Re-epithelialisation was also assessed 14 days post-wounding. The digital wound photographs were de-identified (i.e., participant ID and time-point information removed from the photographs) and the order randomized by a computerized random number generator, to ensure that the dermatologist (P.J.) remained blind to group allocation and time since wounding. The dermatologist rated each wound as “healed” or “not healed”, with healed being defined as complete reepithelialisation of the wound surface. The complete set of photographs was assessed in a
standardized fashion in the same setting twice sequentially, to ensure consistency. All the photographs were then reassessed on a different occasion to determine inter-rater reliability. The Cohen $k$ coefficient was 0.88, indicating high agreement between the two assessments. Where there was a disagreement, the dermatologist reviewed the photograph again to decide on a final rating. Of the 237 photographs assessed, only 8% ($N = 20$) photographs were inconsistently rated as ‘healed’ or ‘not healed’ and needed to be reassessed for a final decision.

Of the 122 participants who completed the study, 10 people did not complete the writing task properly (told the researcher they typed out a children’s book or completed only one or no writing sessions) and were excluded from all analyses (healing, stress, and affect). A further 3 people were excluded from wound analyses due to allergy to the plaster ($N = 1$), or excessive scabbing from bleeding at the wound site obscuring the photograph ($N = 2$). On day 10 a further 12 people were not included in the wound healing analysis because they either missed their appointment ($N = 2$), or the camera malfunctioned ($N = 3$), or the photo had to be taken early due to a public holiday limiting university access ($N = 7$). On day 14, two people did not have their photograph taken due to camera malfunction. There were no significant differences between groups in the number of people excluded for each reason.

**Data analysis**

All data were analysed using the Statistics software package ‘IBM SPSS 19.0 for Windows’. Manipulation checks were conducted using a one-way ANOVA between groups with Bonferroni post-hoc tests for self-reported expressed emotion and LIWC analysis of words used. When data was non parametric Kruskal Wallis $H$ tests were performed. Post hoc Mann Whitney U tests were conducted to look at differences between groups. For the main outcome variable $\chi^2$ tests were performed to compare the association between group allocation and wound healing at both 10 and 14 days. A logistic regression was also performed at day 10. Mixed between-within subjects ANOVA were performed to look at changes over time and between the expressive writing groups on stress and affect. Bonferroni post hoc analyses adjusted for multiple comparisons were performed. Point
biserial correlations were used to look at affect and healing outcomes. For all analyses, a two-tailed alpha level of .05 was used.

Results

Participant attrition and baseline characteristics

Of the 196 individuals who responded to the study advertisements, 9 individuals did not meet the inclusion criteria and 12 withdrew from the study before completing the first questionnaire. Another 46 people requested the participant information sheet but were unable to be contacted to book the first biopsy appointment. A further two participants withdrew after completing the baseline questionnaire and the writing tasks. Six participants could not or did not want to complete the study. In total 122 participants completed the study, 30 of whom were randomized to the emotional disclosure writing task pre biopsy, 30 were randomized to the control writing task pre biopsy, 30 were randomized to the emotional disclosure task post biopsy and 32 were randomized to the control writing task post biopsy (Figure 8).
Responded to study advertisement (N = 204)

Randomized (N = 137)

Completed baseline questionnaire (N = 130)
Declined participation after randomization (N = 7)

Pre biopsy groups: (N = 66)
Allocated to control writing pre-biopsy (N = 33)
Completed writing (N = 33)
Withdraw before biopsy (N = 2)

Allocated to expressive writing pre-biopsy (N = 33)
Completed writing (N = 31)
Withdraw before biopsy (N = 1)

Received biopsy 14 days after baseline (N = 31)
Completed second questionnaire (N = 31)
Withdraw after biopsy (N = 1)

Wound photograph 10 days after biopsy (N = 30)
Healing analysed (N = 26)
Not analysed:
- camera malfunction (N = 1)
- photograph taken early (N = 3)

Wound photograph 10 days after biopsy (N = 30)
Healing analysed (N = 23)
Not analysed:
- photograph taken early (N = 2)
- did not complete writing (N = 1)
- allergy to plaster (N = 1)
- bleeding (N = 2)

Wound photograph 14 days after biopsy (N = 30)
Healing analysed (N = 28)
Not analysed:
- missed appointment (N = 2)
Completed follow-up questionnaire (N = 30)

Post biopsy groups: (N = 64)
Allocated to control writing post-biopsy (N = 33)
Withdraw before biopsy (N = 0)

Allocated to expressive writing post-biopsy (N = 31)
Withdraw before biopsy (N = 0)

Received biopsy 14 days after baseline (N = 31)
Completed second questionnaire (N = 27)
Completed writing (n = 30)
Withdraw after biopsy (N = 1)

Wound photograph 10 days after biopsy (N = 30)
Healing analysed (N = 22)
Not analysed:
- missed appointment (N = 1)
- camera malfunction (N = 1)
- photograph taken early (N = 1)
- did not complete writing (N = 3)
- did not complete writing (N = 3)
- did not complete writing (N = 5)

Wound photograph 14 days after biopsy (N = 30)
Healing analysed (N = 25)
Not analysed:
- did not complete writing (N = 3)
Completed follow-up questionnaire (N = 32)

Wound photograph 14 days after biopsy (N = 30)
Healing analysed (N = 29)
Not analysed:
- did not complete writing (N = 3)
Completed follow-up questionnaire (N = 32)

Wound photograph 14 days after biopsy (N = 30)
Healing analysed (N = 25)
Not analysed:
- did not complete writing (N = 5)
Completed follow-up questionnaire (N = 30)

Excluded (N = 67)
- Decided not to participate/could not commit (N = 12)
- Excluded because smoker (N = 4)
- Under 18 years old (N = 1)
- Did not reply (N = 46)
- Allergic to anaesthetic (N = 1)
- Current inflammatory skin condition or immunologically-related health problem (N = 3)

Figure 8. CONSORT flow diagram.
Of the 122 participants, 119 (98%) completed at least one writing task and 112 (93%) completed all three writing tasks and submitted them online through a secure portal. Participants assigned to the writing task prior to the biopsy were asked to start writing the next day, 13 days before the biopsy appointment, however there were some issues with compliance. On average, participants assigned to write before wounding commenced writing 8 days before the biopsy ($SD = 4.64$), with no significant difference in timing between the control and intervention groups. Likewise, there were some compliance issues with participants assigned to write after wounding, who were asked to start the next day after wounding. On average, those assigned to write after wounding commenced writing 3 days after the biopsy ($SD = 2.32$), with no significant difference in timing between the intervention and control groups. The writing task was completed over 5.98 days on average ($SD = 3.64$), with no significant difference between groups in the number of days over which the writing was completed. Eighty-four (69%) of the 112 participants completed the task over the first 6 days or less as instructed (allowing for one day between writing sessions), with the rest taking 7 or more days to complete the task.

Participant ranged in age from 18 to 41 years (mean = 23.91, $SD = 6.14$). The majority of the sample were female ($N = 94, 71.8$%). Almost half the participants identified themselves as being European ($N = 73, 55.7$%). The rest of the sample identified as being Asian ($N= 47, 35.9$%), Māori or Pacific Island ($N = 12, 9.2$%). Table 5 shows the demographic data and the baseline psychological measures for each group. There were no significant differences between groups on these measures, apart from diet. However, diet was not significantly correlated with healing on Day 10 or Day 14 ($p > .05$).
Table 5. Demographics and baseline characteristics in the four experimental groups.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Control before Mean (SD)</th>
<th>Expressive before Mean (SD)</th>
<th>Control after Mean (SD)</th>
<th>Expressive after Mean (SD)</th>
<th>p</th>
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<td>Demographics</td>
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<tr>
<td>Age</td>
<td>22.24 (4.03)</td>
<td>24.54 (7.60)</td>
<td>24.53 (6.14)</td>
<td>24.32 (6.23)</td>
<td>.463</td>
</tr>
<tr>
<td>Gender:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, N (%)</td>
<td>23 (69.7)</td>
<td>24 (72.7)</td>
<td>25 (73.5)</td>
<td>22 (71.0)</td>
<td>.992</td>
</tr>
<tr>
<td>Men, N (%)</td>
<td>10 (30.3)</td>
<td>9 (27.3)</td>
<td>9 (26.5)</td>
<td>9 (29.0)</td>
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<tr>
<td>Ethnicity:</td>
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<td>NZ European, N (%)</td>
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<td>20 (15.3)</td>
<td>18 (13.7)</td>
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</tr>
<tr>
<td>Asian, N (%)</td>
<td>13 (9.9)</td>
<td>13 (9.9)</td>
<td>12 (9.2)</td>
<td>9 (6.9)</td>
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</tr>
<tr>
<td>Māori and Pacific Island, N (%)</td>
<td>3 (2.3)</td>
<td>1 (0.8)</td>
<td>4 (3.1)</td>
<td>4 (3.1)</td>
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<td>Health Behaviours</td>
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<tr>
<td>No of times alcohol consumed over past three months:</td>
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<td></td>
<td></td>
<td></td>
<td>.793</td>
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<tr>
<td>None, N (%)</td>
<td>6 (18.2)</td>
<td>9 (27.3)</td>
<td>6 (17.6)</td>
<td>7 (22.6)</td>
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</tr>
<tr>
<td>Several times per month, N (%)</td>
<td>17 (51.5)</td>
<td>14 (42.2)</td>
<td>21 (61.8)</td>
<td>17 (54.8)</td>
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<tr>
<td>Several times per week, N (%)</td>
<td>10 (30.3)</td>
<td>10 (30.3)</td>
<td>7 (20.6)</td>
<td>7 (22.6)</td>
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<tr>
<td>Exercise per week:</td>
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<tr>
<td>0-1 times a week, N (%)</td>
<td>3 (9.1)</td>
<td>4 (12.1)</td>
<td>2 (5.9)</td>
<td>1 (3.2)</td>
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</tr>
<tr>
<td>2-4 times a week, N (%)</td>
<td>17 (51.5)</td>
<td>30 (60.6)</td>
<td>16 (47.1)</td>
<td>19 (61.3)</td>
<td></td>
</tr>
<tr>
<td>5-7 times a week, N (%)</td>
<td>13 (39.4)</td>
<td>9 (27.3)</td>
<td>16 (47.1)</td>
<td>11 (35.5)</td>
<td></td>
</tr>
<tr>
<td>Sleep (PSIQ)</td>
<td>5.69 (3.06)</td>
<td>4.96 (2.14)</td>
<td>5.94 (2.83)</td>
<td>5.22 (2.21)</td>
<td>.578</td>
</tr>
<tr>
<td>Diet (rating out of 5; 1= very poor, 5= very good)</td>
<td>3.52 (0.67)</td>
<td>3.64 (0.82)</td>
<td>3.09 (0.71)</td>
<td>3.48 (0.77)</td>
<td>.022</td>
</tr>
<tr>
<td>Psychological measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perceived Stress Scale (PSS)</td>
<td>25.97 (7.37)</td>
<td>25.52 (6.62)</td>
<td>26.21 (6.09)</td>
<td>26.64 (6.09)</td>
<td>.925</td>
</tr>
<tr>
<td>Affect Valuation Index (AVI):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Arousal Positive</td>
<td>7.79 (3.15)</td>
<td>8.24 (2.54)</td>
<td>7.91 (2.85)</td>
<td>7.87 (2.80)</td>
<td>.837</td>
</tr>
<tr>
<td>Positive</td>
<td>8.58 (2.99)</td>
<td>9.42 (2.83)</td>
<td>8.82 (3.35)</td>
<td>9.00 (2.56)</td>
<td>.738</td>
</tr>
<tr>
<td>Low Arousal Positive</td>
<td>11.97 (3.40)</td>
<td>12.67 (3.74)</td>
<td>11.44 (3.62)</td>
<td>11.23 (3.77)</td>
<td>.363</td>
</tr>
<tr>
<td>Low Arousal</td>
<td>9.30 (2.58)</td>
<td>7.91 (2.74)</td>
<td>8.88 (2.41)</td>
<td>8.03 (2.86)</td>
<td>.106</td>
</tr>
<tr>
<td>Low Arousal Negative</td>
<td>7.42 (2.21)</td>
<td>6.39 (2.56)</td>
<td>7.32 (2.50)</td>
<td>6.84 (2.84)</td>
<td>.234</td>
</tr>
<tr>
<td>High Arousal Negative</td>
<td>4.67 (1.61)</td>
<td>4.58 (2.03)</td>
<td>5.59 (2.78)</td>
<td>5.10 (1.94)</td>
<td>.442</td>
</tr>
<tr>
<td>High Arousal</td>
<td>4.58 (2.08)</td>
<td>4.68 (2.34)</td>
<td>4.00 (1.66)</td>
<td>3.97 (1.35)</td>
<td>.391</td>
</tr>
<tr>
<td>Negative</td>
<td>4.85 (2.09)</td>
<td>4.36 (1.67)</td>
<td>5.62 (2.56)</td>
<td>5.00 (2.16)</td>
<td>.184</td>
</tr>
</tbody>
</table>
Manipulation check

A manipulation check was conducted to see whether the average amount of emotion revealed during writing sessions differed between groups. The results are reported in Table 6. In comparison to the two expressive writing groups, the two groups who wrote about a neutral topic reported that they expressed significantly less emotion in their writing, and LIWC analysis showed that they used significantly fewer affective, cognitive, and insightful words, and fewer personal pronouns. There were no significant differences between the two expressive writing groups in the types of words used. There was no significant difference between groups in the frequency of the words ‘biopsy’ and ‘wound’, although participants in the control after group had slightly higher means. This may be because they were asked to write about what they did during the past week and their plans for the upcoming week, which included the receiving the biopsy and attending appointments to get their biopsy dressing changed.
Table 6. Differences between groups in self-reported amount of emotion revealed, as well as LIWC analysis of word use in written essays.

<table>
<thead>
<tr>
<th>Category</th>
<th>Control Before Mean (SD)</th>
<th>Expressive before Mean (SD)</th>
<th>Control after Mean (SD)</th>
<th>Expressive after Mean (SD)</th>
<th>df</th>
<th>F value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emotions revealed (average over 3 sessions)‡</td>
<td>1.52 (0.38)*</td>
<td>4.14 (0.64)†</td>
<td>1.86 (0.76)*</td>
<td>3.99 (0.66)†</td>
<td>3</td>
<td>80.30</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pronouns - 1st person singular (I, me mine; average over 3 sessions)</td>
<td>8.19 (2.38)*</td>
<td>9.55 (2.58)†</td>
<td>7.27 (2.72)*</td>
<td>9.52 (2.75)†</td>
<td>3</td>
<td>5.04</td>
<td>.003</td>
</tr>
<tr>
<td>Cognitions (cause, know, ought; average over 3 sessions)</td>
<td>13.26 (1.92)*</td>
<td>20.09 (2.63)†</td>
<td>13.12 (2.65)*</td>
<td>19.49 (3.00)†</td>
<td>3</td>
<td>62.61</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Insight (think, know, consider; average over 3 sessions)</td>
<td>0.69 (0.49)*</td>
<td>3.55 (1.16)†</td>
<td>1.06 (0.58)*</td>
<td>3.19 (0.94)†</td>
<td>3</td>
<td>87.33</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Positive words (love, nice, sweet; average over 3 sessions)</td>
<td>1.89 (0.86)*</td>
<td>3.08 (1.03)†</td>
<td>1.75 (0.87)*</td>
<td>3.09 (1.08)†</td>
<td>3</td>
<td>16.36</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Negative words (hurt, ugly, nasty; average over 3 sessions)‡</td>
<td>0.43 (0.35)*</td>
<td>3.30 (1.45)†</td>
<td>0.56 (0.44)*</td>
<td>3.80 (2.30)†</td>
<td>3</td>
<td>82.31</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Anxiety words (worried, fearful, nervous; average over 3 sessions)‡</td>
<td>0.10 (0.10)*</td>
<td>0.81 (0.52)†</td>
<td>0.15 (0.17)*</td>
<td>0.88 (0.78)†</td>
<td>3</td>
<td>66.82</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Sad words (crying, grief, sad; average over 3 sessions)‡</td>
<td>0.08 (0.10)*</td>
<td>0.81 (0.68)†</td>
<td>0.13 (0.12)*</td>
<td>0.77 (0.43)†</td>
<td>3</td>
<td>76.82</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Words relating to wound (biopsy, wound; average over 3 sessions)‡</td>
<td>0.01 (0.04)</td>
<td>0.00 (0.01)</td>
<td>0.02 (0.03)</td>
<td>0.00 (0.01)</td>
<td>3</td>
<td>7.38</td>
<td>.061</td>
</tr>
</tbody>
</table>

‡ indicates that non-parametric Kruskal Wallis H test was performed; * indicates that groups are not significantly different from each other; † indicates that groups are not significantly different from each other; difference between * and † p < .001
Primary outcome: Wound re-epithelialisation

There was a significant difference in wound healing at day 10 post-biopsy between writing groups, $\chi^2(3, N = 97) = 8.84, p = .032$. In comparison to the other three groups, participants in the expressive writing before group had a greater proportion of fully reepithelialised wounds: 52% (12/23) of the group healed compared to 15% (4/26) in the control before group, 23% (6/26) in the control after group and 27% (6/22) in the expressive writing after group; Figure 9). This yielded a medium effect size ($\text{Cramer's } V = 0.30$). Column proportion comparisons adjusted for Bonferroni showed that the rate of healing in the expressive writing before group differed significantly from those in both control groups ($p < .05$). Examination of standardised residuals indicated that the high proportion of participants healed in the expressive writing before group (standardised residual = 2.08) contributed to the significant result. The odds of a participant with a healed wound on day 10 were 6.00 times higher if they performed expressive writing before wounding compared to the control writing before wounding group, and 3.64 times higher compared to the control writing after wounding group. A logistic regression showed similar results. The overall model was significant $\chi^2(3, N = 97) = 8.55, p = .036$, Nagelkereke’s $R^2 = .12$. Participants who wrote expressively before wounding were significantly more likely to be healed than participants who wrote about a neutral topic before wounding ($\beta = 1.79, p = .009$), and compared to those who wrote about a neutral topic after wounding ($\beta = 1.29, p = .039$). Participants who wrote expressively after wounding showed no significant difference in healing compared to those who wrote expressively before wounding ($\beta = -1.07, p = .093$), or compared to those who wrote about the neutral topic either before ($\beta = 0.72, p = .318$) or after wounding ($\beta = 0.22, p = .738$).

By Day 14, 80% to 90% of participants in each group achieved full wound reepithelialisation and there were no significant differences between groups, $\chi^2(3, N = 107) = .62, p = .892$. 
The effects of writing before or after wounding on stress and affect

Stress

We were interested in whether the difference in the timing of the expressive writing intervention differentially affected participants' stress and affect over the wound healing period. There were no significant differences in perceived stress between the expressive writing groups at any time point, so this was not considered further. Similarly, there were there no significant changes in stress over time in the two control groups.

Affect

Of the eight subscales assessing affect, there were significant differences between the two expressive writing groups in four domains. First, there was a main effect of time on high arousal affect, $F(2, 82) = 3.72, p = .028$, partial $\eta^2 = .08$, whereby high arousal affect increased during the healing period (from immediately after biopsy to the 14 day follow-up) in both expressive writing groups.
Second, there was a main effect of time \( (F(2, 82) = 7.12, p = .001, \text{partial } \eta^2 = .15) \) and a significant interaction effect between groups over time on low arousal negative affect \( (F(2, 82) = 4.81, p = .011, \text{partial } \eta^2 = .11) \). Bonferroni post hoc analyses found that people who performed expressive writing prior to wounding experienced a significant increase in low arousal negative affect during the writing period (baseline to time of biopsy, \( p = .002 \)); and a decrease in low arousal negative affect during their healing period (immediately after the biopsy to the 14 day follow-up) \( (p < .001) \). People who performed expressive writing after the biopsy appeared to have an increase in low arousal negative affect before the biopsy and the writing task but this was not statistically significant. Similar to the expressive writing before group, they also experienced an increase in low arousal negative affect over the period they wrote (from time of biopsy to follow-up, which coincided with their healing period; Figure 10a). At follow-up, those who completed the expressive writing prior to wounding reported less low arousal negative affect (\( \text{mean} = 6.36, \text{SD} = 2.74 \)) than those who completed the writing post wounding, although this was not statistically significant (\( \text{mean} = 7.71, \text{SD} = 2.15, p = .080 \)).

Third, there was a main effect of time \( (F(1.62, 66.25) = 5.38, p = .011, \text{partial } \eta^2 = .12) \), and a significant interaction effect between groups over time in positive affect \( (F(1.62, 66.25) = 8.01, p = .002, \text{partial } \eta^2 = .16) \). The group who performed expressive writing before wounding experienced a decrease in positive affect over the writing period (baseline to biopsy, \( p = .082 \)), followed by an increase in positive affect during their healing period (from biopsy to follow-up; Figure 10b). Those who performed expressive writing after wounding experienced a significant decrease in positive affect while writing which coincided with their healing period (from biopsy to follow-up, \( p < .001 \)). The results showed that participants who wrote expressively after the biopsy had a significantly larger decrease in positive affect after the writing task (\( \text{mean} = -2.19, \text{SD} = 2.44 \)), compared to the decrease in positive affect in the expressive writing before group (\( \text{mean} = -.77, \text{SD} = 1.48; t(41) = 2.32, p = .026, r = .33 \)). Those who performed expressive writing prior to wounding had significantly
higher positive affect at follow-up (mean = 9.36, SD = 3.59) than those who wrote expressively post wounding (mean = 6.71, SD = 2.80, p = .029).

Fourth, there was a significant interaction effect between the two expressive writing groups over time in high arousal positive affect, \( F(2, 82) = 3.75, p = .028 \), partial \( \eta^2 = .08 \). Figure 10c shows a trend for high arousal positive affect to decrease while writing in the group who wrote before wounding (baseline to biopsy) and to increase during the healing period (from biopsy to follow-up; Figure 10c). The group who performed expressive writing post wounding also experienced a trend for a decrease in high arousal positive affect while writing which coincided with their healing period (from biopsy to follow-up; \( p = .129 \)). At the follow-up point, high arousal positive affect was significantly higher in the group who wrote prior to wounding (mean = 8.68, SD = 3.05) than the group who wrote post wounding (mean = 6.71, SD = 2.32, \( p = .022 \)).

In contrast, there were no significant changes in any affect scales over time for the control groups with one exception. High arousal positive affect increased from the time of biopsy to the follow-up point amongst those who wrote about daily activities after the biopsy (\( p = .004 \)).

**Associations between emotional expression and changes in affect**

To check whether affect ratings were a good proxy for intervention response, correlations were performed between the self-reported amount of emotion expressed during the writing task and affect ratings at day 14. In the expressive writing before group, more emotion expressed on average over the three tasks was correlated with more high arousal positive affect at the 14 day follow-up (\( r_s = .44, p = .042 \)). In the expressive writing after group, more emotion expressed was correlated with less high arousal positive affect at the 14 day follow-up (\( r_s = -.57, p = .005 \)). This suggests that affect did change as a result of emotional expression. It illustrates that soon after completing the writing task, positive affect decreased, but by 14 days after the writing task, sufficient time had passed for it to have a beneficial effect and positive affect increased.
Each cross along the x-axis indicates that one participant performed expressive writing on that day. For example, on day -7, seven participants from the expressive writing before group completed a writing session.
Post hoc analyses

On average, the participants assigned to complete expressive writing after the biopsy started writing 3 days after the biopsy. However, 12 participants started the writing immediately after the biopsy and had completed the writing intervention by day 6. As a post hoc analysis, we investigated whether people who complied with the instructions to write over the first three to six days had better healing than those who took longer to write. There were no significant differences in healing between groups at day 10 ($p = .667$). However, on day 14, 100% (12/12) of the participants who completed the expressive writing task over the first six days were rated as healed, compared to only 67% (8/13) of the participants who took longer to complete the task ($\chi^2(1, N = 25) = 5.77, p = .039, \text{Cramer's } V = 0.48$). The odds of a participant with a healed wound on day 14 were 13.24 times higher if they had completed the writing over the first six days.

We then examined whether expressive writing soon after wounding improved healing at days 10 and 14 compared to the control groups and the expressive writing before group, including only the 12 participants who finished their writing within six days of receiving the biopsy. However, this did not change the significance of the results of the primary outcome, wound re-epithelialisation.

There was a significant negative correlation between taking longer to complete the writing task and high low arousal negative affect at day 14 ($r_s = -.42, p = .039$) within the expressive writing after group. Higher levels of low arousal negative affect at day 14 were also correlated with slower healing on day 14 in this group ($r_{pb} = -.54, p = .005$).

Discussion

This study showed that expressive writing pre-biopsy was associated with a greater proportion of healed wounds by day 10 compared to control writing pre or post biopsy. In contrast, the proportion of healed wounds by day 10 did not differ significantly between the expressive writing post-biopsy group and the two control writing groups. Thus, expressive writing is clearly more efficacious than control writing when performed pre biopsy. However, we also observed that
wound healing between the two expressive writing groups (i.e., those completing writing pre and post biopsy) did not differ significantly. Post hoc analyses indicated there may be differential effects of expressive writing after wounding on healing, depending on the timing of the writing and wound assessment. People who finished the expressive writing task in the first 6 days after the wound had better healing at 14 days than those who took longer to do the expressive writing task after wounding.

Differences in affect between the two expressive writing groups over the healing period may explain why those who performed expressive writing before wounding had better healing. The participants who completed expressive writing before wounding experienced decreases in positive affect and high arousal positive affect, and an increase in low arousal negative affect over the writing period. However, during the healing period, their low arousal negative affect, positive affect and high arousal positive affect scores all rebounded. On the other hand, the participants who completed expressive writing after wounding also experienced decreases in positive affect and high arousal positive affect as well as an increase in low arousal negative affect over the writing period. However this occurred over the same period as healing. In other words, during the healing period, participants who performed expressive writing prior to wounding experienced improvements in positive and negative affect, whereas those who performed expressive writing after wounding experienced deteriorations in positive affect and increases in negative affect, which may have influenced healing. Previous research has linked optimism to better healing (which could be likened to positive affective states), and linked depression with worse healing (which could be likened to negative affective states; Cole-King & Harding, 2001; Ebrecht et al., 2004).

The control groups demonstrated stable affect across the study period, with the exception of the post-biopsy control writing group, who had a significant increase in high arousal positive affect over the time they completed the writing task. In contrast, the post-biopsy expressive writing group had a greater decrease in positive affect after completing the writing task than the pre-biopsy expressive writing group. It is possible that writing after the biopsy had a stronger effect on positive
affect than writing beforehand due to the compound effect of having a wound at the same time. Future research is needed to assess whether this is due to added burden or something else.

The effects of expressive writing do not appear to operate via changes in perceived stress. Similar to this study, previous studies have also found that expressive writing does not reduce perceived stress, but still had an effect on wound healing (Koschwanetz et al., 2013, Weinman et al., 2008). An alternative explanation is that the perceived stress scale used in all three of these studies is not sensitive enough to detect subtle changes in stress or the scale may not assess aspects of stress relevant to expressive writing.

The results suggest that improvements in healing only occur sometime after expressive writing has finished and improvements in affect have begun. Expressing more emotion during the writing task before wounding was associated with greater high arousal positive affect at 14 days after wounding, suggesting that expressive writing can lead to beneficial effects in affect over time (15-28 days). In contrast, expressing more emotion during the writing task after wounding, was associated with lower high arousal positive affect at day 14 after wounding (0-13 days after writing).

Most of the participants did not follow the instructions to write on the assigned days and they tended to write over a spread of days either before or after wounding, which makes the findings in affect more difficult to interpret in relation to writing and healing. However, it appears that expressing emotions initially reduces positive affect and later increases it. Positive affect may aid healing, as is suggested in other work on trait positive affect (Robles et al., 2009). Further evidence supporting the statement that benefits in healing occur only after some time following writing comes from the results of the post-hoc analysis. This showed that participants who finished the expressive writing task in the first six days post-wounding showed better healing on day 14 compared to those who took longer to finish. By completing the writing earlier, participants had more time for their affect to improve after the writing task and on day 14 benefits in healing were seen. Although it has been found that writing over a longer time period is associated with bigger effect sizes for other outcomes (Smyth, 1998), due to the short time period over which healing...
occurred, completing the intervention sooner had better outcomes for participants in this study. It
could be argued that expressive writing both before and after the wound may have some benefits
but it is all in the timing. The beneficial effects of expressive writing on healing seem to emerge after
about 8 -11 days.

Changes to affect may be important during wound repair. Wound healing is composed of
several phases, and research shows that psychological stress affects several components of healing
via enhanced glucocorticoid and catecholamine production (Gouin & Kiecolt-Glaser, 2011). The data
presented in Figure 10 suggest that, on average, there were positive changes in affect in the
expressive writing before group between the time of biopsy to 14 days, which occurred concurrently
with the healing process. For those who wrote after wounding, there were no positive changes to
affect on average during this healing period. However, the affect data is not sufficiently detailed to
be able to match specific changes in affect to specific healing phases more closely.

It is interesting to compare the results of this study to the earlier study on the effects of
timing of relaxation on wound healing (Robinson et al., 2015). Relaxation had beneficial effects on
skin barrier recovery both when performed immediately before or immediately after skin damage
compared to a no-relaxation control group. The mechanism involved may have been the reduction
of physiological arousal and stress hormones, although these were not measured. In contrast to this
study, relaxation was performed within 20 minutes of skin damage, and the wound was more
superficial so healed more quickly. The mechanisms involved in expressive writing are likely to be
different in some ways due the initial distressing effects of expressive writing. However, by the time
this initial distress has dissipated, the resultant effects on stress hormones and inflammatory
responses may be similar. This is an area for future research.

The temporary increases in negative affect that were observed in this study during the
writing intervention have been reported in previous research (Booth et al., 1997). These changes in
affect may be linked to sympathetic activity and release of stress hormones (Miller & Cohen, 2001).
When the distress subsides, there may be a temporary boost in mood and immune parameters.
Supporting this hypothesis, research has found that immediately after expressive writing, participants have lower circulating lymphocytes and basophils in the blood, but one month later these differences disappeared (Petrie, Booth, Pennebaker, Davison & Thomas, 1995). While there is insufficient research at this stage to understand the exact timeframe of changes to affect and immune function after expressive writing, this wound healing study suggests that 10 days may be sufficient time for positive effects on mood and inflammatory processes to occur.

In this study, those who wrote expressively prior to wounding showed changes to both negative affect and positive affect, and both of these aspects may be important to immune function and wound healing. Previous research has shown that negative mood is associated with lower natural killer cell counts assessed across two days at the same (unspecified) time of day (Valdimarsdottir & Bovbjerg, 1997). Furthermore, greater positive mood was associated with higher natural killer cell counts but only in those people who also experienced negative affect. In other words, the experience of positive affect may buffer the effects of negative affect on immune parameters.

Positive health outcomes may be related to decreasing autonomic arousal that occurs as participants engage in emotional processing over time (McGuire, Greenberg & Gevirtz, 2005). By processing events through writing, intrusions associated with the event are less frequent and may decrease the strain on physiological resources required to contend with distressing thoughts (Pennebaker, 1993). The analysis of the words participants used suggests that participants in the expressive writing groups used more cognitive, insightful and affective words compared to participants in the control groups. This suggests (even though the essays were not examined for narrative construction) that participants in the expressive writing groups were able to cognitively process distressing events and previous research suggests that this process can result in physiological improvements (Low, Stanton & Danoff-Burg, 2006).

A further important observation concerns the fact that the majority of wounds had healed by day 14. This, of course, suggests that the effects of the intervention are limited to the speed of
healing, rather than whether or not wounds will heal. We are, however, limited in this study by the nature of the wounds (i.e., small experimental wounds that we would expect to resolve completely). Further examination of this issue would require a focus on chronic wounds where intervention effects on both speed of healing and complete wound resolution could be examined.

This study had a number of limitations. First because the sample was comprised of healthy volunteers, it is difficult to apply these finding to clinical settings. Further research needs to replicate these findings in clinical populations, such as people with chronic wounds or people undergoing surgery. The longer time period needed for chronic wounds to heal may mean that expressive writing could improve healing even though it is performed after the wound has occurred. Another limitation is that this study did not measure stress hormones, such as cortisol, during the writing tasks. This might be useful for understanding the underlying biological processes involved in the effects of expressive writing on healing and to help determine optimal timing of intervention delivery. The measurement of circulating or in situ immune cells or cytokines would add further physiological evidence for these effects. In order to understand the effects of writing on affect and healing better, future research should measure affect more frequently. Due to the limited assessment points, it may not be appropriate to attribute changes in affect to wound healing efficacy, and more research is needed in this area. However, other research measuring affect after performing expressive writing have shown similar changes (Booth et al., 1997). Lastly, a mediation model testing whether affect mediated the effects of writing on healing was not tested due to multiple time points and groups, and mediational modelling is an area for future research.

Conclusions

This research supports the literature showing that expressive writing during the two week period prior to a punch biopsy improves wound healing in comparison to neutral writing. Expressive writing after wounding may also improve healing, but the effects appear dependent on the timing of the writing and the healing assessment point. This research highlights the complexities of implementing an expressive writing intervention and the importance of timing for wound healing.
The results suggest that expressive writing causes initial distress followed by improvements in affect which may affect healing. However, further research is needed to assess the effects of expressive writing on wound healing and affect using more time points, and to investigate effects in clinical populations, before stronger conclusions can be made.
Chapter 7: Expressive writing influences wound healing:

Preliminary immunohistochemistry analysis of skin tissue two weeks after punch biopsy wounding.

Preface

Rationale

Chapter 6 reported the results of a study examining the effects of timing of an expressive writing intervention on wound healing. Participants were randomised into one of four groups, where they wrote either emotionally about past traumas or neutrally about daily events, before or after receiving a 4mm punch biopsy wound. Healing was assessed via ratings of re-epithelialisation.

Chapter 7 reports the preliminary findings of the second part of this study, which involved an immunohistochemical analysis of the healing skin as an alternative way to assess healing. The rationale for investigating immune parameters within the tissue sample was to get an objective physiological measure of healing to corroborate the surface rating. Ratings of wound healing progress in photographs have generally been considered the best method of healing assessment because it allows for non-invasive, standardised measurements (Dyson et al., 2003; Koschwanez & Broadbent, 2011). However, since the healing of the wound underneath the surface is not visible, and the majority of healing occurs in the wound bed, photographs may not allow for an accurate assessment. Research has also used ultrasound scanning at a way of assessing the structural tissue changes within the wound bed (Ebrecht et al., 2004; Maple et al. 2015) although ultrasound cannot record the appearance of the wound. Furthermore, research has also analysed biological markers, such as in wound fluid (Glaser et al., 1999; Kiecolt-Glaser et al., 2005), but have not looked at immune cell changes in the tissue during the healing process. This should be considered given previous research has found different patterns of immune cell activation in healthy tissue samples in people who report higher levels of stress (Koschwanez et al., 2015). To date, no studies have
taken a second punch biopsy over the top of a partially healed punch biopsy wound to assess tissue healing, as done in the current research.

It was originally hypothesized that participants randomised to perform expressive writing either prior to or after wounding would have more macrophages, neutrophils, T-cells, Langerhans cells and immune cell activation in the healing tissue than the control groups, indicative of a better healing wound. However, to be consistent with the re-epithelialisations results, those randomised to the group who performed expressive writing before wounding would have more macrophages, neutrophils, T-cells, Langerhans cells and immune cell activation than all the other groups.

To present, immunohisotchemical analyses have been performed on only 48 of the 122 samples sent to Professor John Tarlton, a biochemist at The University of Bristol for analysis on July 31st 2015. For the purposes of this thesis, the results have been written as a draft paper, and the results of the additional samples will be added before the paper is sent for publication. It was expected that the whole analysis would take approximately six months but it has taken much longer (1 year and 4 months so far) due to technical difficulties with tissue orientation.

**Aims**

1. To explore whether an expressive writing intervention completed either before or after a 4mm biopsy could influence immunohistochemical markers in the healing wound, compared to writing about a neutral topic.

2. To assess whether these immunohistochemical variables in healing wounds were correlated with ratings of epithelialisation.

**Citation**

Abstract

Objectives: In a previous study, expressive writing performed before a 4mm punch biopsy wound resulted in better healing than writing neutrally either before or after wounding, as assessed by ratings of surface re-epithelialisation at 10 days. This subsequent study aimed to analyse the biological mechanisms behind these effects by conducting an immunohistochemistry analysis of the healing tissue, 14 days after wounding.

Methods: A 5mm punch biopsy was taken 14 days after the original 4mm wound over the same site. Forty-eight of the 5mm biopsy tissue samples were randomly selected from the original sample of 122 participants (12 participant samples from each of the four allocated groups) for the initial immunohistochemistry analysis. The skin tissue samples from the 5mm biopsy were tested for the number of macrophages, neutrophils, T-cells, Langerhans cells and immune cell activation.

Results: Participants who performed expressive writing before wounding had significantly more epidermal Langerhans cells in the healing tissue than whose who performed neutral writing after wounding ($p = .024$). Participants who performed expressive writing after wounding also had significantly more epidermal Langerhans cells in the healing tissue than those who completed the control task after the biopsy ($p = .016$). Differences between groups in macrophages, neutrophils, T-cells, and immune cell activation were not significant.

Conclusions: Expressive writing either prior to after wounding increases the number of Langerhans cells in the healing skin. Langerhans cells are involved in the first-line defense of the epidermal barrier and are important in the inflammatory phase of acute wound healing. They migrate from the wound site to lymphoid organs, and are replenished during the proliferation phase of healing. This supports previous results showing that expressive writing can speed epithelialisation, and provides evidence of one biological mechanism. It provides evidence that expressive writing after wounding can have beneficial effects on cellular mechanisms.
Introduction

Written emotional disclosure (expressive writing) is an intervention whereby people write for 20 minutes a day for three consecutive days about their deepest thoughts and emotions regarding a previous traumatic event or stressor (Pennebaker, 1993). It is thought that expressive writing helps people to create a narrative structure for the traumatic event, stimulating cognitive processing and reducing negative affect when later recalling the event (Pennebaker, Mayne, & Francis, 1997). Expressive writing has been found to be beneficial not only in terms of self-reported health, psychological well-being and general functioning (Smyth, 1998), but also immunological function. For example, compared to a control writing task, writing emotionally about past traumatic events resulted in significantly higher antibody level 6 months after a hepatitis B vaccine (Petrie et al., 1995), greater lymphocyte proliferation after mitogen stimulation (Pennebaker, Kiecolt-Glaser & Glaser, 1988) and increased CD4+ lymphocyte counts and lower viral loads in human immunodeficiency virus (HIV) patients (Petrie, Fontanilla, Thomas, Booth & Pennebaker, 2004).

Recent research has found that expressive writing performed during the two weeks prior to a punch biopsy wound can improve healing (Koschwanez et al., 2013; Weinman et al., 2008), implicating alterations in the immune system and healing process. However, to date these studies have assessed healing either via ratings of surface re-epithelialisation (Koschwanez et al., 2013) or ultrasound of the base of the wound (Weinman et al., 2008). No studies have investigated the cellular processes that occur as a result of writing in the healing wound. When injury occurs, effective repair of a wound requires communication between many different cells types, and various cells have different functions within this process associated with host defense, inflammation and regulation of the healing process (Park & Barbul, 2004; Reinke & Sorg, 2012). Initially, neutrophils and monocytes arrive at the wound site (Davies, Jenkins, Allen & Taylor, 2013). Neutrophils are the first responders and play an important part in phagocytosis and wound debridement. After neutrophils, monocytes migrate to the wound site and differentiate into tissue resident macrophages or dendritic cells. The role of macrophages during the inflammation phase of
healing is to help promote inflammation by producing cytokines and chemokines that attract leukocytes, remove neutrophils after the early stages of inflammation, and promote cell proliferation and protein synthesis (Koh & DiPietro, 2011; Landén, Li & Ståhle, 2016). Macrophages are important in preventing infection due to expression of Major Histocompatibility Complex (MHC) class II proteins that help the immune system to recognize foreign substance through the human leukocyte antigen (HLA) system, which encodes the MHC proteins in humans (Goldsby et al., 2003). Macrophage phagocytosis of exogenous bacteria or proteins, leads to intracellular digestion to peptides. These peptides are then coupled to MHC II molecules and exported to the cell surface, where MHC/peptide can interact with specific CD4 and CD3 T-cells to drive an adaptive immune response to any infection. Macrophages can also have an anti-inflammatory role, by decreasing immune activation by releasing anti-inflammatory cytokines (such as IL-10) and growth hormones (TGF-β, VEGF and insulin-like growth factor-1; Koh & DiPietro, 2011). Langerhans cells are dendritic cells located in the epidermis (Cumberbatch, Dearman, Griffiths & Kimber, 2000). Their role is to act as immune sentinels recognising and processing antigens. During inflammation, Langerhans cells migrate from the skin to lymph nodes (Stoitzner, Tripp, Douillard, Saeland & Romani, 2005). Langerhans cells are then repopulated by circulating monocytes after inflammation has subsided (Collin & Milne, 2016; Merad, Ginhoux & Collin, 2008; Romani, Tripp & Stoitzner, 2012). Gamma-delta T-cells (which express CD3), which make up 50% of T-cells in the dermis (Anane et al., 2009), contribute to wound healing by producing epithelial growth factors and inflammatory cytokines (Carding & Egan, 2002; Girardi, 2006; Jameson, et al., 2002) and protection against infection (Nakasone et al., 2007; Wang, Kamath, Das, Li & Bukowski, 2001). Research shows that healing is impaired when gamma-delta T-cells are not present in the skin (Havran & Jameson, 2010).

Psychological stress, negative affective states, and distress have been found to affect cellular immunity via a number of pathways (Herbert & Cohen, 1993; Segerstrom & Miller, 2004). Specifically, psychological stress can impact glucocorticoid responses resulting in a failure to down-regulate the inflammatory response altering circulating plasma levels of neutrophil and lymphocytes
(Cohen et al., 2012). Furthermore, stress has been found to alter the proliferation, cytokine secretion, and trafficking of macrophages (Padgett & Glaser, 2003), mobilization of gamma-delta T-cells (Anane et al., 2010) and reduce Langerhans cell frequencies in the epidermis (Hosoi et al., 1998; Kleyn et al., 2008). Finally, the stress of surgery or trauma can down-regulate HLA expression, compared to a control group (indicating reduced immune cell activation), leading to post-operative complications (Wakefield, Carey, Foulds, Monson, & Guillou, 1993).

Previous research has found that immune cells in the skin are related to stress and linked to healing. Koschwanez et al., (2015) reported that stress was associated with fewer macrophages in the skin of older adults. In addition, stress was associated with lower activation of immune cells in the skin (as measured by HLA expression) in a sample of younger adults. Furthermore, reduced HLA expression, and fewer Langerhans cells were associated with slower healing (Koschwanez et al., 2015).

Recently a randomised controlled trial was conducted by the authors investigating the effects of expressive writing and its timing on the healing of a 4mm punch biopsy wound to the upper arm. Using a random number generator participants were randomised to four different groups; expressive writing prior to the original biopsy, neutral writing prior to the original biopsy, expressive writing after the original biopsy and neutral writing after the original biopsy. Re-epithelialisation was assessed by a dermatologist blinded to group allocation. Expressive writing improved re-epithelialisation rates 10 days after the wound, but only in the group who wrote expressively prior to wounding compared to the two control groups (Robinson, Jarrett, Vedhara & Broadbent, 2017). While those who performed expressive writing after wounding showed no significant differences in healing compared to the other groups at 10 days, those who wrote in the first 6 days after wounding had better healing at 14 than those who wrote on later days. This suggested that writing after wounding may be effective depending on the timing of writing and assessment.
The aim of this follow-up study was to assess how expressive writing impacted cells in the healing skin using immunohistochemical analysis. This may provide a more sensitive measure of physiological effects of expressive writing than examining surface re-epithelialisation. A 5mm punch biopsy was taken over the site of the original biopsy 14 days after the initial wound and the removed tissue was analysed by immunohistochemistry to investigate the cellular population of the healing skin. It was hypothesized that participants in the expressive writing groups would have increased numbers of Langerhans cells, macrophages, neutrophils, T-cells and greater HLA immune cell activation, indicative of faster healing. We also hypothesised that these cells would be associated with ratings of epithelialisaton.

Methods

Sample

Participants were recruited from the university campus and local community by email, online advertisements and flyers. To take part in the study participants had to be healthy (no skin allergies or immunological-related health problems) aged between 18 and 55 years and able give written informed consent. Furthermore if participants were pregnant, or were a smoker, they were not able to participate. Ethics approval was obtained from The University of Auckland Human Participants Ethics Committee (UAHPEC). Permission was obtained for storage of human tissue at The University of Bristol from the UK Human Tissue Authority.

Funding was available for immunohistochemical analysis of 96 samples. The samples were randomly selected from those participants who had wounds that were assessed for re-epithelialisation from photographs at 14 days. Immunohistochemical analysis was initially performed on a subset of 48 participants from the total sample of these 96 participants and the results are presented here. To select the participants to be included in this analysis, 12 participants from each of the four groups were randomly selected using a random number generator. The list was re-ordered randomly before it was given to those performing the immunohistochemical analysis to ensure they remained blind to group allocation.
Procedure

Study procedures are reported elsewhere (Robinson et al., 2017) and will be described in brief. Figure 11 provides an overview of the study timeline. Two weeks prior to the initial wounding appointment, participants were contacted by phone or email and screened for eligibility and performed the baseline questionnaire. To randomise participants into groups, a researcher uninvolved in the project created a random list using a random number generator. After informed consent and receiving the baseline questionnaire, the researcher opened a sealed opaque envelope revealing to which group the participant was allocated. Participants were randomised to one of four groups: expressive writing task pre wounding, control writing task pre wounding, expressive writing task post wounding and control writing task post wounding. Those allocated to write pre wounding were then given the intervention instructions online and asked to complete the writing task over the next three days before their appointment 14 days later. Those allocated to write after wounding were told they should perform the writing task over the first three days after wounding. They were told it would be acceptable to skip a day in between each writing session if they were unable to write on three consecutive days.

At the first appointment participants received a 4mm punch biopsy wound located on the inner arm, 7cm proximal to the medial epicondial of the humerus. The procedure was performed by a dermatologist. Before the procedure, the area was cleaned with an alcohol solution. The area of the upper arm was then anaesthetized using 1% lignocaine and 1:200,000 adrenaline. The wound was photographed using an EOS 100D Canon camera (Canon Ltd., Tokyo, Japan) with a Canon Ultrasonic EF 100-mm f/2.8 Macro USM lens and Canon ringflash. The wound was then dressed with DuoDERM Extra Thin hydrocolloid dressing (ConvaTec, Skillman, NJ) and a water-proof plaster (Cutifilm Plus; Smith & Nephew, London, UK). Participants were told not to remove the hydrocolloid dressing until the next appointment, but could change the plaster as needed. After the appointment those allocated to the post-biopsy writing groups were instructed to complete the online writing task over the next three days.
Ten days after the original biopsy wound, participants were scheduled for a follow-up appointment. At this appointment the wound area was cleaned with sterile saline and gauze, photographed, and a new hydrocolloid dressing and plaster was applied. Fourteen days after the original biopsy participants returned for another appointment, where the second 5mm biopsy was taken from exactly the same area as the first biopsy. This biopsy was taken so that the healing tissue from the first wound could be removed and analysed for immunohistochemical markers. This timepoint was chosen because the dermatologist taking the biopsies was only available for one day a fortnight. Fourteen days represents a timepoint in the healing process when inflammation is subsiding and proliferation or growth of new tissue is occurring. Prior to this second biopsy, the wound was cleaned and photographed. The biopsy procedure was conducted using the same protocol as previously. The second biopsy diameter was 1mm larger to ensure that the entire wound site was removed. Immediately after the procedure had been performed the tissue sample was embedded in a tin foil mould filled with FSC22 Clear Frozen Section Compound (Leica Biosystems, Melbourne), and frozen with liquid nitrogen. The sample was then stored at -80°C until analysis. The biopsy site was dressed with a plaster. Participants were given a $40 voucher as compensation for their time.
Responded to study advertisement

Randomized to study group

Pre biopsy writing groups

Performed neutral writing prior to biopsy

Received 4mm punch biopsy wound

Wound photograph 10 days after biopsy

Wound photograph 14 days after biopsy

Received 5mm punch biopsy for analysis

N = 28

Randomly selected for analysis N = 24
Analyzed N = 12
Undergoing analysis N = 12

Performed expressive writing prior to biopsy

Received 4mm punch biopsy wound

Wound photograph 10 days after biopsy

Wound photograph 14 days after biopsy

Received 5mm punch biopsy for analysis

N = 30

Randomly selected for analysis N = 24
Analyzed N = 12
Undergoing analysis N = 12

Post biopsy writing groups

Received 4mm punch biopsy wound

Wound photograph 10 days after biopsy

Wound photograph 14 days after biopsy

Wound photograph 14 days after biopsy

N = 30

Received 4mm punch biopsy wound

Wound photograph 10 days after biopsy

Wound photograph 10 days after biopsy

Wound photograph 14 days after biopsy

N = 32

Received 5mm punch biopsy for analysis

N = 30

Randomly selected for analysis N = 24
Analyzed N = 12
Undergoing analysis N = 12

Figure 10. Flow chart of study timeline for each group
Expressive writing intervention

The expressive writing task followed a standardised script used in previous studies (Korschwanez et al., 2013; Pennebaker, 1993; Weinman et al., 2008). Participants randomised to complete the expressive writing task, either before or after the biopsy were asked to write about their “deepest thoughts and feelings about a traumatic, upsetting experience of your entire life.” The instructions stated that if they could not think of a traumatic experience they should write about a significant life-changing event. Participants were asked not to write about something they had discussed in great detail with someone else.

The control group writing instructions asked participants to write about how they spent their time. For the first session they were asked to write about the past week, for the second session they were asked to write about the past 24 hours and for the last session they were asked to write about their plans for the upcoming week. Participants were asked to keep their writing free from emotions and only write about the facts.

Upon receiving the writing instructions, regardless of group allocation, participants were asked to start writing the next day and write at home for 20 minutes a day, over 3 consecutive days. They were told not to worry about spelling or grammar and if they missed a day to continue with the writing task the following day. Participants used a secure online portal to complete the writing tasks, as this has been effectively used in previous research (Sheese et al., 2004). To ensure anonymity, each participant was given an individual code so they could log on to the portal to complete each writing task and submit it for analysis. The writing was saved so that it could be analysed the Linguistic Inquiry and Word Count (LIWC; Pennebaker et al., 2007) computer programme, which categorizes text into multiple psychologically relevant categories (Pennebaker & Francis, 1996). Participants were reminded daily by email or text to complete the writing task each day. At the end of each writing session, participants were asked to report how much emotion they revealed, ranging from 1 (“not at all”) to 5 (“a great deal”) (Pennebaker et al., 1990), to check that participants followed the writing instructions.
Measures

Demographic and psychological measures

Participants were asked their age, weight, height, ethnicity and education level. Health behaviour data were collected regarding alcohol consumption, smoking status, exercise regularity, and diet. Alcohol consumption was rated over the past 3 months from 1 (never) to 6 (everyday). On days participants did drink they were asked to rate how many drinks they had ranging from 1 (0 drinks) to 7 (12 or more drinks). Participants were asked to rate how often they did physical activity for 30 minutes over an average week from 1 (never) to 8 (everyday). They were asked to rate their diet over the past week from 1 (very poor) to 5 (very good). Sleep was also assessed using the Pittsburgh Sleep Quality Index (PSQI; Buysse et al., 1989). The scale consists of 19 questions that are totalled to give an overall global score. The scale demonstrated good internal reliability in this sample (Cronbach’s α = .70). Demographic measures and health behaviours were assessed at baseline, 2 weeks prior to the first biopsy.

Immunohistochemistry assessment

The primary outcome for this paper was the analysis of cells in the second 5mm punch biopsy. This outcome shows the numbers of different cells present in the healing skin 14 days after the initial wound for all of the groups. Frozen tissue samples were shipped by courier on carbon dioxide at -80 C to The University of Bristol, UK. to ensure the samples remained frozen. Immunohistochemical staining was performed on the skin samples embedded in freezing medium (FSC22 Clear Frozen Section Compound; Leica Biosystems, Melbourne) using mouse anti CD207 (langerin) antibody (clone 306G9, Novus Biologicals Europe, Abingdon, UK) to identify Langerhans cells in epidermis; mouse anti-CD68 (clone KP1, AbD Serotec Bio-Rad Laboratories, Inc., Hercules, CA) to identify macrophages; mouse anti-human leukocyte antigen HLA (cloneHL-39, AbD Serotec) to establish level of immune cell activation; mouse anti-CD3 (clone UCHT1, AbD Serotec) and mouse anti-myeloperoxidase (MPO) (clone 2C7, AbD Serotec ) to detect neutrophils. Non-specific antibody binding was blocked with goat serum before adding primary antibodies. Primary antibodies were
detected with isotype specific biotin conjugated secondary antibodies (Jackson Immunoresearch Labs Inc., West Grove, PA). Secondary antibodies were followed by Strepavidin-biotin-horseradish peroxidase conjugates (Vector Labs). Antibody localization was performed using a peroxidase reaction with H₂O₂ and 3,3-diaminobenzidine (DAB) tetrahydrochloride (Sigma Aldrich, St. Louis, MO) as the chromogen.

Cellular infiltration for CD3, HLA, MPO, CD68 and CD207 expressing cells was assessed subjectively across the entire section using the criteria in Table 7. The scorer was blinded to group allocation.

**Data analysis**

All data were analysed using the Statistics software package ‘IBM SPSS 19.0 for Windows’. Immunohistochemistry data were checked for normality, resulting in Kruskal Wallis tests being performed for all immunohistochemistry outcomes. Mann-Whitney U tests were performed post hoc to test for differences between the expressive writing before group and the other three groups. Correlations were performed to examine relationships between immunohistochemistry outcomes.

**Results**

**Baseline Characteristics**

Of the 48 participants included in this initial immunohistochemistry analysis, the age range was 18 to 41 years (Mean = 24.35, SD = 6.32). The majority of the sample were female (N = 34, 71%). Just over half the participants identified themselves as being European (N = 28, 58%). The rest of the sample identified as being Asian (N= 13, 27%), Māori or Pacific Island (N = 7, 15%). Table 8 shows the demographic data and the baseline psychological measures for each group. There were no significant differences between groups on these measures. There were no significant differences between this sub sample of 48 participants and the overall sample on any demographic variables, health behaviours or perceived stress at baseline.
Table 7. Subjective scoring for tissue sections in x 20 objective field.

<table>
<thead>
<tr>
<th>Score</th>
<th>CD3, HLA, MPO cells/field</th>
<th>CD68 cells/field</th>
<th>CD207 (epidermis only) Keratinocyte (Kc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;10, sparse distribution</td>
<td>&lt;50, sparse distribution</td>
<td>10 Kc intervals between single positive cells</td>
</tr>
<tr>
<td>2</td>
<td>10-100, well scattered cells and up to 3 small clusters</td>
<td>50-100 in &lt;30% tissue</td>
<td>Equal mix of 1-3Kc and 10Kc intervals between clusters of 1-3 positive cells</td>
</tr>
<tr>
<td>3</td>
<td>&gt;100, up to 30% tissue area infiltrated, small and large cell clusters</td>
<td>100-200 in 30-50% tissue</td>
<td>&gt;80% epidermis has 1-3Kc intervals between frequent clusters of 1-10 positive cells</td>
</tr>
<tr>
<td>4</td>
<td>30-50% tissue infiltrated, many large clusters</td>
<td>&gt;200 in &gt;50% tissue, presence of dense clusters</td>
<td>&gt;3 areas of continuous positive cells (10+), with 3-10Kc intervals</td>
</tr>
<tr>
<td>5</td>
<td>100% tissue infiltrated at all depths, dense distribution of positive cells</td>
<td>100% tissue infiltrated at all depths, dense distribution of positive cells</td>
<td>Nearly continuous positive cells, 1-3 intervals of 1-3Kc</td>
</tr>
</tbody>
</table>
Table 8. Demographics and baseline characteristics in the four experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>Control Before Mean (SD)</th>
<th>Expressive before Mean (SD)</th>
<th>Control after Mean (SD)</th>
<th>Expressive after Mean (SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>23.00 (5.13)</td>
<td>26.08 (8.12)</td>
<td>24.75 (7.31)</td>
<td>23.58 (3.96)</td>
<td>.656</td>
</tr>
<tr>
<td>Gender:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.569</td>
</tr>
<tr>
<td>Women, N (%)</td>
<td>7 (58)</td>
<td>9 (75)</td>
<td>10 (83)</td>
<td>8 (67)</td>
<td></td>
</tr>
<tr>
<td>Men, N (%)</td>
<td>5 (42)</td>
<td>3 (25)</td>
<td>2 (17)</td>
<td>4 (33)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.361</td>
</tr>
<tr>
<td>NZ European, N (%)</td>
<td>4 (33)</td>
<td>10 (83)</td>
<td>7 (58)</td>
<td>7 (58)</td>
<td></td>
</tr>
<tr>
<td>Asian, N (%)</td>
<td>5 (42)</td>
<td>2 (17)</td>
<td>2 (17)</td>
<td>4 (33)</td>
<td></td>
</tr>
<tr>
<td>Māori and Pacific Island, N (%)</td>
<td>3 (25)</td>
<td>0 (0)</td>
<td>3 (25)</td>
<td>1 (8)</td>
<td></td>
</tr>
<tr>
<td><strong>Health Behaviours</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of times alcohol consumed over past three months:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.371</td>
</tr>
<tr>
<td>None, N (%)</td>
<td>1 (8)</td>
<td>1 (8)</td>
<td>3 (25)</td>
<td>2 (17)</td>
<td></td>
</tr>
<tr>
<td>Several times per month, N (%)</td>
<td>8 (67)</td>
<td>6 (50)</td>
<td>7 (58)</td>
<td>6 (50)</td>
<td></td>
</tr>
<tr>
<td>Several times per week, N (%)</td>
<td>3 (25)</td>
<td>5 (42)</td>
<td>2 (17)</td>
<td>4 (33)</td>
<td></td>
</tr>
<tr>
<td>Exercise per week:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.287</td>
</tr>
<tr>
<td>0-1 times a week, N (%)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>2-4 times a week, N (%)</td>
<td>8 (67)</td>
<td>9 (75)</td>
<td>9 (75)</td>
<td>5 (42)</td>
<td></td>
</tr>
<tr>
<td>5-7 times a week, N (%)</td>
<td>4 (33)</td>
<td>2 (17)</td>
<td>3 (25)</td>
<td>7 (58)</td>
<td></td>
</tr>
<tr>
<td>Sleep (PSIQ)</td>
<td>5.36 (2.54)</td>
<td>5.09 (1.81)</td>
<td>6.10 (1.79)</td>
<td>5.18 (2.64)</td>
<td>.733</td>
</tr>
<tr>
<td>Diet (rating out of 5; 1= very poor, 5= very good)</td>
<td>3.67 (0.67)</td>
<td>3.50 (0.80)</td>
<td>3.00 (0.60)</td>
<td>3.75 (0.75)</td>
<td>.452</td>
</tr>
<tr>
<td><strong>Psychological measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perceived Stress Scale (PSS)</td>
<td>27.42 (7.41)</td>
<td>24.00 (5.26)</td>
<td>26.17 (4.71)</td>
<td>26.17 (6.70)</td>
<td>.589</td>
</tr>
</tbody>
</table>
Manipulation check

Based on participants’ ratings of the amount of emotion they expressed after each writing sessions, participants followed the instructions given. Compared with the two control writing groups those in the expressive writing groups rated their essays as more emotional (control before Mean = 1.58 (SD = 0.43); control after Mean = 1.58 (SD =0.45); expressive writing before Mean = 4.31 (SD = 0.39); expressive writing after Mean = 4.08 (SD = 0.64). This was statistically significant, $H(3) = 35.97$, $p < .001$).

Primary outcome: Immunohistochemistry results

**Macrophages, neutrophils and T- cells**

Table 9 shows that all groups had similar levels of macrophages, neutrophils and T-cells in the epidermis and dermis based on the mean categorical scores for each field. Kruskal Wallis test showed there was no significant difference between groups in macrophages, $X^2(3, N = 48) = 0.59$, $p = .900$, macrophages and neutrophils, $X^2(3, N = 48) = 1.42$, $p = .702$, T-cells in the dermis, $X^2(3, N = 48) = 1.82$, $p = .611$, epidermis base, $X^2(3, N = 48) = .18$, $p = .981$, or mid epidermis, $X^2(3, N = 48) = 3.32$, $p = .345$.

**Langerhans cells**

A Kruskal Wallis test showed there was a significant difference between groups in Langerhans cells, $X^2(3, N = 48) = 7.83$, $p = .050$ (see Table 9). Post hoc tests found that the expressive writing before group had significantly more Langerhans cells than the control after group ($p = .024$). The expressive writing after group also had significantly more Langerhans cells than the control after group ($p = .016$). There were no other significant post hoc tests.

In order to see if there was an overall effect of expressive writing on Langerhans cells the groups were combined into those that completed the expressive writing versus those that did the control writing task regardless of timing. The results were significant, $U = 185.00$, $z = -2.24$, $p = .025$, indicating that those in the expressive writing groups had higher levels of Langerhans cells than the control groups.
**Immune cell activation (HLA expression)**

A Kruskal Wallis test showed there was no significant difference between groups in immune cell activation, \( \chi^2(3, \, N = 48) = 1.86, \, p = .603 \). Again, to see if there was an overall effect of expressive writing regardless of timing, the groups were combined into those who completed the expressive writing versus those that did the control writing task. The results were not significant, \( U = 227.50, \, z = -1.30, \, p = .192 \), with more immune cell activation in the expressive writing groups.

**Associations between immune cells and ratings of re-epithelialisation**

Spearman correlations were performed with all groups combined to see if there were any significant relationships between macrophages, neutrophils, T-cells, and Langerhans cells. The results showed there was a significant positive correlation between macrophages (CD68) and neutrophils and macrophages \( r_s = .52, \, p < .001 \). Langerhans cells were significantly negatively correlated with neutrophils and macrophages (Myeloperoxidase) \( r_s = -.29, \, p = .049 \).

From the photographs taken on day 10, wounds were more likely to be rated as healed if there were fewer neutrophils and macrophages (Myeloperoxidase) in the tissue sample \( r_s = -.30, \, p = .042 \) on day 14. From the photographs taken on day 14, wounds were more likely to be rated as healed if there were more T-cells in the epidermis (CD3) in the tissue sample at day 14, \( r_s = .30, \, p = .044 \). There was not a significant correlation between Langerhans cells and ratings of healing on day 10 \( r_s = .15, \, p = .303 \) or day 14 \( r_s = .12, \, p = .443 \).
Table 9. Comparisons of macrophages, neutrophils, T-cells, Langerhans cells and immune cell activation between groups.

<table>
<thead>
<tr>
<th></th>
<th>Control writing before group, (N = 12)</th>
<th>Expressive writing before group, (N = 12)</th>
<th>Control writing after group, (N = 12)</th>
<th>Expressive writing after group, (N = 12)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Macrophages dermis (CD68)</td>
<td>3.33 (0.62)</td>
<td>3.13 (0.80)</td>
<td>3.08 (1.00)</td>
<td>3.25 (0.87)</td>
<td>.900</td>
</tr>
<tr>
<td>Neutrophils and macrophages dermis (Myeloperoxidase)</td>
<td>2.63 (0.77)</td>
<td>2.01 (0.84)</td>
<td>2.25 (1.14)</td>
<td>2.17 (1.03)</td>
<td>.702</td>
</tr>
<tr>
<td>Immune cell activation dermis (HLA)</td>
<td>2.92 (0.67)</td>
<td>3.29 (0.78)</td>
<td>2.83 (1.05)</td>
<td>3.08 (0.90)</td>
<td>.603</td>
</tr>
<tr>
<td>T-cells dermis (CD3)</td>
<td>2.50 (0.48)</td>
<td>2.46 (0.89)</td>
<td>2.71 (0.92)</td>
<td>2.29 (0.62)</td>
<td>.611</td>
</tr>
<tr>
<td>T-cells epidermis base (CD3)</td>
<td>2.00 (0.95)</td>
<td>2.08 (0.79)</td>
<td>2.17 (1.03)</td>
<td>2.17 (1.11)</td>
<td>.981</td>
</tr>
<tr>
<td>T-cells mid epidermis (CD3)</td>
<td>1.67 (0.78)</td>
<td>1.50 (0.90)</td>
<td>1.25 (0.62)</td>
<td>1.67 (0.89)</td>
<td>.345</td>
</tr>
<tr>
<td>Langerhans cells epidermis (CD207)</td>
<td>1.75 (0.72)</td>
<td>2.21 (1.08)</td>
<td>1.29 (0.45)</td>
<td>1.83 (0.54)</td>
<td>.050</td>
</tr>
</tbody>
</table>
Discussion

This paper examines whether expressive writing performed either before or after wounding affects the number of different cell types in the healing skin, compared to neutral writing. For all four groups, the cells numbers were assessed 14 days after the initial wounds (by taking a second biopsy of the healing skin at 14 days). The findings suggest that expressive writing results in higher levels of Langerhans cells in the epidermis of healing skin at 14 days compared to neutral writing. People who performed expressive writing either before or after the original wound had higher levels of Langerhans cells, compared to people who wrote about a neutral topic after wounding. This suggests expressive writing resulted in better healing parameters regardless of writing timing.

These findings lend support to the results reported in the previous paper on ratings of re-epithelialisation from the larger sample (Robinson et al., 2017). In that paper, participants assigned to write expressively prior to receiving a punch biopsy were more likely to be rated as healed based on re-epithelialisation than participants in the two control groups, although there were no differences between those who wrote expressively after wounding and the other three groups (Robinson et al., 2017). However, in this paper there were significant differences between the people who wrote after the initial biopsy and those who wrote about a neutral topic after the biopsy. The reason why the expressive writing after group had more Langerhans cells in the tissue compared to the control after group, but did not have higher rates of re-epithelialisation, may be because re-epithelialisation is faster than healing under the epidermis and it takes time for the benefits of expressive writing to occur. An alternative explanation may be that the measure of Langerhans cells was more sensitive than ratings of re-epithelialisation so there was more power to detect effects. In addition, by chance 8/12 randomly selected for immunohistochemical analysis completed their writing over the first six days after wounding, which was associated with better healing in the total sample (Robinson et al., 2017).

These results also support previous research implicating the role of Langerhans cells in wound healing. Research examining the healing of diabetic foot ulcers (Stojadinovic et al., 2013),
reported that higher number of Langerhans cells in the epidermis correlated with healing. Other research found that participants who had lower levels of stress had more Langerhans cells in their skin (Koschwanez et al., 2015). Furthermore, faster wound healing between days 14 and 21 was associated with more Langerhans cells in the skin (Koschwanez et al., 2015).

It is interesting that no significant differences were seen in other immunohistochemistry results. This may be because the biopsy of the original biopsy was taken 14 days after the original wound. The process of healing would have moved beyond the initial inflammatory response, when neutrophils would be abundant in the wound (Landén et al., 2016). If neutrophils remain at the wound site for longer, they can be detrimental to healing (Dovi, Szaderska & DiPietro, 2004). The removal of neutrophils is important to move into the proliferative phase of healing and macrophages are responsible for removing neutrophils in the wound by inducing neutrophil apoptosis (Meszaros, Reichner & Albina, 2000). In this study, there was an association between healing on day 10 and lower levels of macrophages and neutrophils indicating that healing was moving to the proliferation phase. Furthermore, lower levels of neutrophils were associated with higher levels of Langerhans cells.

A limitation of this research is the small number of samples in the analysis. This study was conducted with healthy volunteers, which means that the results presented cannot be applied to clinical settings, such as those with chronic ulcer wounds. More research needs to be conducted in clinical populations to determine how expressive writing may influence immune parameters. Another limitation is that the tissue sample was taken two weeks after the initial wound was created. This means that the changes between groups during the inflammatory phases, which occurs in the first few days after healing, could not assessed. Future research should consider taking biopsies of wounded tissue at different stages throughout the healing process. This may provide valuable information about the effects of psychological interventions on cellular infiltration and healing.
In conclusion, these preliminary findings suggest that expressive writing either before or after wounding can increase the number of Langerhans cells in the healing skin at 14 days, supporting the effectiveness of the intervention for improving healing. Further investigation is needed to support these preliminary findings.
Chapter 8: The role of social closeness during tape stripping to facilitate skin barrier recovery: Preliminary findings

Preface

Rationale

This thesis has investigated the effects of timing of two psychological interventions (relaxation and expressive writing) on wound healing. Another kind of intervention identified in the systematic review (Chapter 3) as requiring further research for effects on healing was social support. Social support has been extensively studied in regards to how it can influence physical health outcomes (Berkman, Glass, Brissette & Seeman, 2000; Uchino, 2009), and research shows that people with low levels of social support have higher mortality from cardiac disease (Ell, Nishimoto, Medianski, Mantell & Hamovitch, 1992), cancer and other illnesses (Lee & Rotheram-Borus, 2001).

The systematic review identified three studies that looked at social support and wound healing but the three studies differed markedly. Notably, the sources of social support in each intervention were different. In Robles (2007) study one-off support was offered by a confederate who was not subjected to the same stress. In Edwards et al., (2009) study, support came from being part of an ongoing group with similar types of wounds. Finally in Ginandes et al., (2003) study ongoing support was provided by a professional who was able to provide answers around what to expect after surgery and practical information. In clinical settings, it is difficult to know what type of support a person needs, particularly if they are about to undergo a potentially stressful procedure. The current research was undertaken after finding in the review that there are a variety of types of support and considering how support from a similar other may influence wound healing.

The final experimental study (Study 3) presented in this chapter draws on the idea that support from people who are undergoing a similar procedure or are feeling similar levels of stress may be helpful in terms of providing informational and emotional support (Kulik, Maher & Moore,
Again, the question of timing is important, as social support may be more useful prior to wounding, so that anxiety around the procedure is reduced and the person is in a better psychological state for improved healing. This study explored whether having a similar other present during a wounding procedure and during recovery, and with whom you had a chance to talk to before the procedure affected wound healing. Although this study did not explicitly alter the timing of the intervention between groups, the findings indicate that being paired with a similar other has a beneficial effect on healing. The findings cannot be directly applied to clinical populations, but they indicate the potential for research to study the effects of social support from others about to undergo or who have undergone a similar procedure on healing.

The systematic review conducted in Chapter 3 identified that even though stress slows wound healing due to activation of the hypothalamic-pituitary-adrenal axis, resulting in increased levels of cortisol, few studies measured cortisol to investigate whether alterations in cortisol secretion mediated benefits in wound healing. In Chapter 3, only one social support intervention measured cortisol (Robles, 2007), and it is questionable whether they successfully provided social support for participants as they did not report any reductions in anxiety compared to participants that received no social support. However, other research indicates that higher perceived levels of social support are known to decrease cortisol levels (Eisenberger et al., 2007; Turner-Cobb, Sephton, Koopman, Blake-Mortimer & Spiegel, 2000). Based on the literature showing that stress can impede healing and disrupt physiological and immunological processes, and social support may help reduce stress, it is important to look at changes in physiological measures of stress to identify a physiological basis via which psychological interventions may operate.

Study 3 is a randomised controlled trial conducted with a healthy population, designed to assess whether social support can influence skin barrier recovery. It was hypothesized that participants randomised to perform a social closeness induction task with a participant they did not know and remain paired with them throughout the study period would show faster skin barrier recovery compared to those assigned to a control group. It was also hypothesized that they would
report less self-reported stress, have smaller physical stress responses and show greater reductions in autonomic nervous system activation as evidenced in salivary cortisol and alpha-amylase compared to the control group.

**Aims**

1. To investigate whether being paired with another participant for a social closeness induction task before a tape stripping procedure and remaining paired with this person throughout a tape stripping procedure and during the recovery period could improve skin barrier recovery in a sample of healthy participants compared to a control group who complete the study procedure alone.

2. To explore whether this interaction could influence salivary cortisol and alpha-amylase concentrations compared to the control group.

**Citation**

Abstract

Objectives: Social support is known to reduce the negative effects of stress on health however there is mixed evidence for the effects of social support on wound healing. This study aimed to investigate whether undergoing a task designed to promote social closeness with a fellow participant and being paired with that person during a tape stripping procedure could reduce stress responses and improve skin barrier recovery compared to going through tape stripping alone.

Methods: Seventy-two healthy adults were randomised to either a social closeness condition where participants completed a relationship building task and tape stripping in pairs, or a control condition where they completed tape stripping alone. Skin barrier recovery was measured using trans-epidermal water loss. Salivary cortisol and alpha-amylase were collected at four time points as markers of the endocrine and autonomic stress response.

Results: Social closeness had a beneficial effect on skin barrier recovery compared to the control condition ($t(54) = 2.86, p = .006, r = .36$). Social closeness also significantly reduced self-reported stress over the study session. The effects of the intervention on skin barrier recovery were moderated by self-reported stress reduction ($p = .035$). Analyses of stress biomarkers found no significant differences in cortisol between groups but alpha-amylase increased significantly more from baseline to after tape stripping in the control group compared to the intervention group.

Conclusions: This is the first study to show that social closeness with a person going through a similar unfamiliar procedure can positively influence wound healing. Future research needs to replicate these findings in other wound types and in clinical settings.
Introduction

Research has shown that psychological stress can have a negative impact on wound healing, which includes the slowing of skin barrier recovery after a skin tape stripping procedure (Altemus et al., 2001; Walburn et al., 2009). Tape stripping causes damage to the epidermis, which elicits a subsequent cascade of immunological events to repair the damage (Gouin & Kiecolt-Glaser, 2011). Stress can slow skin barrier recovery due to an increase in endogenous glucocorticoids (cortisol) that suppress epidermal lipid synthesis (Choi et al., 2005; Garg et al., 2001, Robles, 2007) and reduce the proliferation of cytokines necessary to help restore skin barrier function (Nickoloff & Naidu, 1994; Tsai et al., 1994; Wood et al., 1997).

Recent studies have investigated whether psychological interventions to reduce stress can benefit wound healing. The strongest evidence to date is for relaxation (Holden-Lund, 1988; Broadbent et al., 2012; Robinson et al., 2015) and expressive writing interventions (Koschwanez et al. 2013; Weinman et al., 2008) to buffer the stress response and promote healing. Another promising intervention is social support.

Social support is a heterogeneous concept, which can be seen as having two broad components - structural and functional (Holt-Lunstad & Uchino, 2015). Structural support describes the extent to which individuals are involved in social networks (e.g. being married, or number of social contacts) whereas functional support refers to actual or perceived supportive social interactions (e.g. the provision of emotional, informational, tangible, or belonging support). Both components of support have demonstrated beneficial effects on health (Holt-Lunstad & Uchino, 2015; Uchino et al., 1996). For example, social support can lower mortality rates in cardiac disease (Barth et al., 2010) and cancer (Aizer et al., 2013).

Interventions to improve social support have been varied, with a review of 100 studies categorising supportive interventions as group or individual, as providing direct support or building social skills, and as provided by a peer (friend or person facing the same problem) or by a professional (Hogan, Linden & Najarian, 2002). The review found that social support interventions
were generally beneficial, including different types of support and different populations (patients with HIV, cancer, rheumatoid arthritis, depression and addiction issues; Hogan et al., 2002).

Several theories have been proposed to explain why social support can improve health. The social support reactivity hypothesis states that supportive others exert their effects on health by preventing or attenuating physiological responses to stress (Lepore, 1998). Studies testing this hypothesis can be divided into those that have used an active support paradigm and those that have used a passive support paradigm (Lepore, 1998). Active support refers to a person actively providing supportive comments or gestures, whereas passive support refers to the mere presence of someone familiar, a peer, or a non-evaluative person. Both passive support and active support have been shown to reduce physiological activity during stressful tasks (Kamarck, Manuck, & Jennings, 1990; Lepore, 1998). Research has indicated that passive support can be as effective as active support at reducing pain during a cold pressor test (Brown et al., 2003).

Social comparison theory suggests that when people are facing a threat, they like to affiliate with others who are facing the same threat, possibly to provide emotional comparison, emotional support, cognitive clarity, and to reduce anxiety (Schachter, 1959). Cardiac surgery patients have shorter hospital stays when they have a post-surgery roommate or a cardiac surgery roommate prior to the operation than when they have no roommate (Kulik et al., 1996). Other research has shown that talking with fellow patients in a shared room results in better perceived health, fewer activity limitations and lower psychological stress post-surgery amongst patients undergoing coronary artery bypass (Thoits, Hohmann, Harvey & Fletcher, 2000). However, this research did not assess wound healing as an outcome.

There have been only two intervention studies to date on the effects of social support on wound healing with mixed results. First, patients randomised to receive wound care in a community setting with other patients where peer support and social interaction were encouraged, had faster healing of chronic leg ulcers compared to patients who received wound care from nurses during home visits (Edwards et al., 2009). The community setting group also reported increased positive
social interactions, but not greater social support overall, than the home visit group. Second, laboratory-based research showed that the presence of a unfamiliar confederate, providing emotional, instrumental, informational, and validation support during preparation for a stressful task, did not reduce stress and did not improve skin barrier recovery after tape stripping, (Robles, 2007). This suggests the presence of an unfamiliar person may not provide the same benefits as the presence of a peer.

Related work has shown that social relationships can have both positive and negative aspects and the quality of a relationship is an important factor in its effects on health and healing (Robles, Slatcher, Trombello, & McGinn, 2013; Kiecolt-Glaser et al., 2005). Blister wounds healed more slowly when couples underwent a conflict resolution task than when they underwent a more supportive task (Kiecolt-Glaser et al., 2005). Furthermore, couples undergoing a socially supportive task showed that those who had higher oxytocin levels (a hormone released during social bonding) during the task had more positive communication behaviours and faster wound healing than those with lower oxytocin levels (Gouin et al., 2010).

One problem with conducting experiments with naturally occurring relationships is that established couples vary in their histories of satisfying and unsatisfying interactions, which can have a confounding influence on results (Sedikides, Campbell, Reeder & Elliot, 1999). Therefore a paradigm has been developed to experimentally induce close relationships (Aron, Melinat, Aron, Vallone, & Bator, 1997; Sedikides, et al., 1999). Close relationships have been shown to be characterised primarily by self disclosure, as well as support, shared activities, and explicit expression of the value of the relationship (Parks & Floyd, 1996). The relationship induction task is designed to develop a short-term relationship through structured self-disclosure. This task has been found to improve closeness between participants (Sedikides, Campbell, Reeder & Elliot, 1998) and increase disclosure of personal information (Liu, Guastella & Dadds, 2012).

Consistent with the social support reactivity hypothesis, research suggests that close relationships reduce autonomic nervous system activation, cortisol release, and inflammation, via
the hypothalamic-pituitary-adrenal axis (Heinrichs, Baumgartner, Kirschbaum & Ehlert, 2003; Kiecolt-Glaser, Gouin & Hantsoo, 2010). It is therefore important to measure both self-reported stress and physiological measures of the stress response as potential mechanisms in the effects of social support on healing. Cortisol can be easily and reliably assessed in saliva (Hellhammer, Wüst & Kudielka, 2009). Similarly, alpha-amylase, a digestive enzyme secreted from pancreas and saliva glands, has been found to be associated with norepinephrine and indicative activation of the autonomic nervous system in response to stress (Rohleder, Nater, Wolf, Ehlert & Kirschbaum, 2004). Alpha-amylase can be measured without difficulty in saliva (Chatterton, Vogelsong, Lu, Ellman & Hudgens, 1996).

This study aimed to investigate whether the presence of a peer with a close relationship could improve wound healing compared to being alone. We used an experimental design, in which participants were randomised either to a control group, where they underwent the experiment alone, or to a social closeness condition. In the social closeness condition, participants were paired with another participant in the same room prior to the tape stripping procedure and underwent a brief social closeness induction task for 15 minutes (Sedikides et al., 1999). The pair of participants remained together during and after the tape stripping procedure. Skin barrier recovery was the primary outcome. The study also aimed to assess potential mechanisms, by assessing self-reported stress as well as cortisol and salivary alpha-amylase as secondary outcomes and markers of hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system arousal (Chida & Steptoe, 2009; Nater & Rohleder, 2009).

It was hypothesized that those in the social closeness condition would have faster skin barrier recovery, lower self-reported stress, lower cortisol and lower alpha-amylase. Furthermore, we hypothesised that those people in the social closeness condition who liked their partner more would have better healing than those who liked their partner less.
Methods

Sample

Participants were recruited from the local community and university campus through flyers, email and online advertisements. Participants had to be over the age of 18 years and provide written informed consent. Exclusion criteria were allergies to adhesive tape, an inflammatory dermatological condition, or taking immunosuppressive medication affecting the immune system (for example, prednisone). Data collection took place between November 2015 and January 2016. Participants attended appointments starting at 1.30pm or 3.30pm. This was to ensure that participants were not completing the task in the morning, when cortisol levels are known to peak after waking (Fries, Dettenborn & Kirschbaum, 2009). Ethics approval was obtained from The University of Auckland Human Participants Ethics Committee (UAHPEC).

Previous research showed peer support reduced ulcer area with an effect size of $d = .61$ (Edwards et al., 2009). Using an effect size $d = .60$, with power set at .80 and a two tailed significance level of $\alpha = .05$, the total sample size required for a $t$ test analysis was calculated using G*Power to be 72 (36 participants per group; Faul et al., 2007).

Procedure

Participants took part in a 2 hour session at The University of Auckland Clinical Research Centre in a ventilated room, where the temperature was controlled by the central university heating system. In order to control the humidity of the room a dehumidifier was set to reduce humidity levels to between 40-60% (average temperature 26°C, average humidity 48%). These conditions were consistent with the guidelines for operating the TEWLmeter probe recommended by the manufacturer. The TEWLmeter probe is a small wand-like instrument designed to measure density gradient water evaporation from the skin (evaporation rate in $g/h/m^2$) using two pairs of sensors (temperature and relative humidity) inside a hollow cylinder. Participants were asked not to shower or exercise for half an hour before the session or apply moisturiser to their arms. In accordance for
salivary sampling, participants were also asked not to eat or brush their teeth within an hour of the appointment, chew gum 24 hours before the appointment, or drink caffeine, juice or alcohol 18 hours before the appointment. On enrolment participants were randomised to one of two groups: control or social closeness. In order to match participants for the social closeness condition randomisation had to occur once the participant confirmed enrolment in the study. Participants were gender matched. Randomisation was performed by a researcher uninvolved in this project using a random number generator. The researcher delivering the intervention and performing the tape stripping was therefore not blinded to the condition of the participant. The researcher followed a standardised written script for both groups explaining the study protocol and tape stripping procedure. The researcher was instructed not to communicate with the participant beyond reading the instructions or answering direct questions. The researcher spent the same amount of contact time with each group.

Both conditions followed a strict protocol. On arrival to the lab after obtaining consent all participants completed a baseline questionnaire relating to health behaviours, sleep, stress levels and current pain. They were then asked to rest for 30 minutes, after which the first saliva sample was obtained. During this time participants were provided with magazines to read and were instructed not to use any communication devices (cell phones, laptops, etc). They were not in the same room as the researcher. After this period, participants were informed which group they had been randomised to and those in the social closeness group met the other person (another study participant of the same sex) and completed a 15 minute relationship building task (RCIT) according to standardised protocol (Sedikides et al., 1999). The researcher left the room while participants completed the task. Those in the control group were asked to read for 15 more minutes instead. After this period all participants completed another brief measure about current stress, and the second saliva sample was collected. Skin barrier disruption was then performed on all participants using a standardised tape stripping procedure. After skin barrier disruption was completed a third saliva sample was collected, as well as a brief measure on current stress. Participants were told they
had to wait 30 minutes until recovery could be measured, and instructed not to touch the tape stripping site. Participants in the control condition again read quietly during this time. In the social closeness condition, the researcher pre-determined which participant was to complete the tape stripping procedure first based on which person enrolled in the study first. Once the first participant completed the tape stripping procedure they remained in the same room while the second participant completed the tape stripping procedure. Participants were able to freely communicate with one another, but communication with the researcher was limited. The researcher did not communicate other than giving instructions and describing the tape stripping procedure. At the end of the session, before skin barrier recovery was tested all participants were asked to provide a final salvia sample and complete the last measure about current stress. Upon completion of the study, all participants received a $20 gift voucher.

**Relationship Induction Closeness Task (RCIT)**

Participants assigned to the social closeness condition completed a conversation task (Relationship Closeness Induction Task, RCIT; Sedikides et al., 1999) with another participant. Participants were matched to a person of the same sex. The task has 29 questions that participants asked one another over a 15 minute period designed to facilitate social closeness. The questions progressively became more personal. The first list consisted of 6 questions to be completed over 2 minutes (example questions included “What is your name” and “Where are you from”). The second list consisted of 12 questions to be completed over 5 minutes (example questions included “If you could change one thing about yourself, what would that be?” and “If you could have one wish granted, what would it be?”). The last list consisted of 10 questions to be completed over 8 minutes (example questions included “If a crystal ball could tell you the truth about yourself, your life, the future or anything else, what would you want to know?”). Participants took turns answering each question first.
Measures

Tape stripping and skin barrier recovery

The tape stripping has been used previously experimental research investigating the influence psychological variables on skin barrier recovery (Garg et al., 2001; Robles, 2007). Skin barrier function was measured by obtaining readings of transepidermal water loss (TEWL) using a TEWLmeter (Courage Khazaka, Germany) at four $1\text{cm}^2$ sites $1\text{cm}$ below the elbow crease on the inner arm. The reading indicates the skin’s ability to prevent water loss from the epidermis. Higher TEWL indicates that more water loss is occurring through the skin and skin barrier function is decreased.

Prior to the tape stripping procedure the TEWLmeter probe was placed in a probe heater set to $34^\circ\text{C}$. This was to ensure that the sensors inside the probe were close to skin temperature and an accurate and reliable reading could be obtained. To take the baseline measures participants were instructed to keep their arm flat and still on a cushion on a desk while the measurement was taken. The TEWLmeter measured each site for 90 seconds, taking a measurement each second.

The three test sites were then dry shaved with a disposable razor to ensure that no hair was pulled out during tape stripping, leaving one control site unaffected. Standard strapping tape (Elastoplast™ 3.8cm x 10m) was applied 10 times to the three test areas. Each strip was gently pushed on to the arm and removed. After 10 strips the first site was tested to determine whether the skin barrier had been disrupted by a minimum of $15\text{ g/h/m}^2$ above baseline. If not, another 10 strips were applied to the test area and it was tested again. Tape was applied until the skin had been elevated by at least $15\text{ g/h/m}^2$ or the maximum of 40 strips of tape had been applied to the skin. A measurement for each site including the control site was taken to determine the elevated level. The entire procedure took approximately 20 minutes to complete. For the rest of the session participants kept their arm uncovered and were instructed not to touch the area. At the end of the session, 30 minutes later, the skin was measured again using the TEWLmeter. Results from previous studies
indicate that the greatest increase in healing occurs within the first hour (Robles, 2007; Robles et al., 2009). Faster return to baseline TEWL levels indicates faster healing.

**Transepidermal water loss analysis**

Twenty consecutive measurements with a standard deviation below 0.5 were averaged to give an overall TEWL reading for each site and time-point. Skin barrier recovery was computed as a percentage based on the formula used in several studies:

\[
\text{Recovery} = \frac{\text{TEWL}_{\text{elevated}} - \text{TEWL}_{\text{recovery}}}{\text{TEWL}_{\text{elevated}} - \text{TEWL}_{\text{baseline}}} \times 100\%. \quad (\text{Robles, 2007; Robles et al., 2009}).
\]

There was some variation in TEWL readings which may have been due to the room being ventilated rather than climate controlled (Miteva et al., 2006). Data were screened to ensure that only accurate and valid data were included in the analysis. This was conducted in accordance with previous research (Robinson et al., 2015). One participant had high TEWL readings at baseline (over 20 g/h/m², which was over 2 SD above the mean, \(M = 14.14, SD = 2.36\)) and was therefore removed from the analysis. Outlying sites were removed if the skin was not sufficiently impaired (elevated by 5 g/h/m², \(N = 2\)). Sites were then averaged if they all showed similar TEWL measurements as result of tape stripping (within 10 g/h/m²). Otherwise, the closest two sites were averaged as per Robles (2007). If the remaining sites were not within 30 g/h/m² of each other the readings were excluded because this indicates a reliable reading was not obtained (\(N = 13\)). After exclusion, there were 29 participants in the control condition and 27 in the social closeness condition (see Figure 12).
Responded to study advertisement (N = 90)

Excluded (N = 18)
- Decided not to participate (N = 3)
- Could not commit to session (N = 14)
- Excluded due to skin condition (N = 1)

Randomized (N = 72)

Allocated control group (N = 36)
- 15 minutes quiet reading
- Tape stripping and TEWL assessment
- 30 minute recovery period
- TEWL assessment

Included in final analysis (N = 29)
- Excluded
  - Not elevated enough (N = 2)
  - Variance in readings (N = 5)

Allocated social closeness group (N = 36)
- 15 minute conversation task
- Tape stripping and TEWL assessment
- 30 minute recovery period
- TEWL assessment

Included in final analysis (N = 27)
- Excluded
  - Baseline too high (N = 1)
  - Variance in readings (N = 8)

Figure 11. CONSORT flow diagram.
**Demographic and psychological measures**

Participants were asked about their age, weight, height, ethnicity and education level, as well as questions relating to health behaviour. Body mass index (BMI) was calculated based on self-reported height and weight. Frequency of alcohol consumption was assessed over the past 3 months from 1 (“never”) to 6 (“everyday”), and the amount on those occasions was assessed from 1 (“0 drinks”) to 7 (“12 or more drinks”). Participants were asked to rate how often they did physical activity for 30 minutes over an average week from 1 (“never”) to 8 (“everyday”). They were asked to rate their diet over the past week from 1 (“very poor”) to 5 (“very good”).

Sleep was assessed with questions adapted from the Pittsburgh Sleep Quality Index (PSQI; Buysse et al., 1989). Participants were asked their waking times, as well as how long it took them to get to sleep the previous night and the amount of sleep they had over the last 24 hours. They were also asked to rate the quality of their sleep last night from 1 (“very good”) to 4 (“very bad”) and how tired they felt today from 1 (“very tired”) to 4 (“not tired at all”). They also rated their sleep quality over the past week from 1 (“very poor”) to 5 (“very good”).

Participants rated their stress levels over the past four weeks using the 10 item Perceived Stress Scale (PSS; Cohen & Williamson, 1988), in which they indicated how often they felt a certain way on a scale from 0 (“never”) to 4 (“very often”). This scale showed strong internal reliability (Cronbach’s $\alpha = .84$). Participants also completed a visual analogue scale (VAS) of their current stress level on a 100 mm line. For stress the anchors at each end were 0 (“not stressed at all”) to 10 (“extremely stressed”). Participants were given this scale at four time points: baseline, before tape stripping, after tape stripping, and recovery.

After completing the relationship closeness induction task participants in the social closeness group completed a 10 item questionnaire asking how they found the task. Six of the questions were in a “yes/no” format, including questions such as “Do you consider conversation a good way to get to know somebody?” The other four questions asked participants to rate their feelings towards their partner on a scale from 1 to 9. This included questions about perceptions of
closeness, liking, similarity and future friendship, where higher scored indicated positive feelings towards their partner. Previous research has found that this task is successful in eliciting social closeness and altering physiological measures (Brown et al., 2009). In this study this scale showed good reliability (Cronbach’s $\alpha = .80$).

**Salivary cortisol and salivary alpha-amylase**

For determination of salivary cortisol and alpha-amylase, four saliva samples were obtained (at baseline, before and after tape stripping, and 30 minutes after tape stripping ended) as per protocol using SaliCaps® collection devices (IBL, Hamburg, Germany). Before obtaining the saliva sample participants rinsed their mouth with water, were asked to swallow and then were told not to swallow for the next two minutes. They then transferred the cumulated saliva via a straw into a SaliCap® collection tube. Saliva samples were stored at -20°C and shipped on dry ice to the Biochemical Laboratory of the Department of Clinical Biopsychology, University of Marburg, Germany. Saliva samples were again stored at -20°C until biochemical analysis. After thawing, saliva samples were centrifuged at 3000 rpm for 11 min. Salivary cortisol concentrations were determined using commercial enzyme-linked immunosorbent assay (ELISA; IBL, Hamburg, Germany). Alpha-amylase activity was determined in diluted saliva samples (1:400 using 0.9% saline solution) by using a kinetic colorimetric test with reagents from Roche (Roche Diagnostics, Mannheim, Germany). The reagents contain the enzyme alpha-amylase and alpha-glucosidase, which convert the substrate ethyliden nitrophenyl to p-nitrophenol. The rate of formation of p-nitrophenol is directly proportional to the amylase activity. The activity is determined by measuring the absorbance at 405 nm (Spectrostar nano, BMG Labtech, Ortenberg, Germany). Inter- and intra-assay coefficients of variation for both assays were below 10%.

**Data analysis**

All data were analysed using the Statistics software package ‘IBM SPSS 19.0 for Windows’.

An independent t test was conducted to assess differences in skin barrier recovery between groups. This was followed by ANCOVAs to control for known covariates (age, number of strips of tape, level
of skin barrier impairment) that were associated with skin barrier recovery, and perceived stress at baseline. To assess the impact of the closeness task on self-reported stress, cortisol, and alpha-amylase, mixed ANOVAs we performed to determine changes over time in both groups. ANCOVAs on changes in cortisol and alpha-amylase controlling for baseline were also conducted for reactivity (from baseline to after tape stripping) and recovery (from after tape stripping to follow-up). For cortisol and alpha-amylase, area under the total response curve with respect to increase (AUC) was calculated using the trapezoid formula following Pruessner et al. (2003). The cortisol and alpha-amylase data was checked for normal distribution and since it was non-parametric a log10 transformation was applied in order to approximate normal distribution. In the alpha-amylase analysis five samples were excluded because they were below the detection limit. Finally, correlations were conducted to see whether changes in biological measures were associated with skin barrier recovery.

Results

Demographics

Seventy-two people participated in this research, aged between 18 and 55 years ($M = 24.67$, $SD = 9.54$). The majority of the sample was female (55 females) and the sample consisted of participants identifying as Asian (Chinese, Korean, Indian and Sri Lankan; 73%), or “other” (New Zealand European, American or European; 27%). Most participants had recently completed a university degree or diploma ($N = 42$; 56%) or had recently completed secondary school and were currently studying towards a degree ($N = 28$; 39%). The remaining sample had completed a trade or technical certificate ($N = 4$; 5%) or a university diploma ($N= 3$; 2%).

Table 10 shows there were no significant differences between groups in demographic variables, health behaviours, salivary measures or psychological variables at baseline. When baseline data for only those participants who were included in the TEWL analysis were examined, there were still no significant differences between the control group and the intervention group in demographic variables, health behaviours, salivary measures or psychological variables at baseline (Table 10).
There were no significant differences in these variables between those participants included in the TEWL analysis and those who were excluded.

Table 11 shows the TEWL data for those included in the primary TEWL analysis. There were no significant differences between groups in baseline TEWL, the mean number of strips applied, or elevation in TEWL after stripping. Wilcoxon Signed Rank tests showed that the control site (not stripped) was elevated significantly less than the disrupted sites showing that the tape stripping did impair skin barrier ($z = -6.51, p < .001, r = -.62$). There were no significant differences in the control site readings between groups at baseline, mid-point or recovery, $p > .05$. Of those participants with valid TEWL readings ($N = 56$), there were no significant differences between groups on demographic or psychological variables at baseline.
Table 10: Difference in baseline variable between control and intervention groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (all participants; N=36)</th>
<th>Intervention (all participants; N=36)</th>
<th>p value</th>
<th>Control (included in TEWL analysis; N=29)</th>
<th>Intervention (included in TEWL analysis; N=27)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender- Females (N, % female)</td>
<td>27 (75%)</td>
<td>28 (78%)</td>
<td>.781</td>
<td>21 (72%)</td>
<td>22 (79%)</td>
<td>.422</td>
</tr>
<tr>
<td>Age (Mean, SD)*</td>
<td>25.83 (10.89)</td>
<td>23.50 (7.96)</td>
<td>.446</td>
<td>27.00 (11.81)</td>
<td>23.33 (6.79)</td>
<td>.377</td>
</tr>
<tr>
<td>Ethnicity (N, %)</td>
<td></td>
<td></td>
<td>.224</td>
<td></td>
<td></td>
<td>.233</td>
</tr>
<tr>
<td>Asian</td>
<td>25 (69%)</td>
<td>28 (78%)</td>
<td></td>
<td>21 (72%)</td>
<td>21 (78%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>11 (31%)</td>
<td>8 (22%)</td>
<td></td>
<td>8 (28%)</td>
<td>6 (22%)</td>
<td></td>
</tr>
<tr>
<td><strong>Health Behaviours</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (Mean, SD)*</td>
<td>22.09 (4.11)</td>
<td>22.80 (3.43)</td>
<td>.248</td>
<td>21.63 (3.00)</td>
<td>23.04 (3.86)</td>
<td>.130</td>
</tr>
<tr>
<td>No of times alcohol consumed over past three months</td>
<td></td>
<td></td>
<td>.785</td>
<td></td>
<td></td>
<td>.993</td>
</tr>
<tr>
<td>None (N, %)</td>
<td>8 (22.2%)</td>
<td>9 (25%)</td>
<td></td>
<td>6 (21%)</td>
<td>6 (22%)</td>
<td></td>
</tr>
<tr>
<td>Several times per month (N, %)</td>
<td>22 (61.1%)</td>
<td>21 (58.3%)</td>
<td></td>
<td>20 (69%)</td>
<td>16 (59%)</td>
<td></td>
</tr>
<tr>
<td>Several times per week (N, %)</td>
<td>6 (16.7%)</td>
<td>6 (16.7%)</td>
<td></td>
<td>3 (10%)</td>
<td>5 (19%)</td>
<td></td>
</tr>
<tr>
<td>Exercise per week</td>
<td></td>
<td></td>
<td>.577</td>
<td></td>
<td></td>
<td>.213</td>
</tr>
<tr>
<td>0-1 times a week (N, %)</td>
<td>2 (5.5%)</td>
<td>4 (11.2%)</td>
<td></td>
<td>2 (7%)</td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>2-4 times a week (N, %)</td>
<td>23 (63.9%)</td>
<td>25 (69.4%)</td>
<td></td>
<td>19 (66%)</td>
<td>21 (78%)</td>
<td></td>
</tr>
<tr>
<td>5-7 times a week (N, %)</td>
<td>11 (30.6%)</td>
<td>7 (19.4%)</td>
<td></td>
<td>8 (27%)</td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>Past 24 hours sleep (hours)</td>
<td>7.96 (1.36)</td>
<td>7.76 (1.53)</td>
<td>.553</td>
<td>7.84 (1.39)</td>
<td>7.88 (1.55)</td>
<td>.916</td>
</tr>
<tr>
<td>Quality of sleep last night (rating out of 4; 1= very good, 5= very bad)*</td>
<td>1.81 (.75)</td>
<td>1.89 (.67)</td>
<td></td>
<td>1.83 (.66)</td>
<td>1.93 (.68)</td>
<td>.993</td>
</tr>
<tr>
<td>Diet (rating out of 5; 1= very poor, 5= very good)</td>
<td>3.31 (.82)</td>
<td>3.31 (.89)</td>
<td>.493</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salivary cortisol (nmol/l)*</td>
<td>4.89 (5.12)</td>
<td>4.63 (2.63)</td>
<td>.489</td>
<td>4.29 (5.01)</td>
<td>4.69 (2.85)</td>
<td>.207</td>
</tr>
<tr>
<td>Salivary alpha-amylase (U/ml)*</td>
<td>66.02 (79.48)</td>
<td>68.25 (92.11)</td>
<td>.617</td>
<td>65.81 (78.52)</td>
<td>73.77 (103.70)</td>
<td>.616</td>
</tr>
<tr>
<td><strong>Psychological measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perceived Stress Scale (Mean, SD)</td>
<td>15.19 (6.78)</td>
<td>16.78 (4.70)</td>
<td>.253</td>
<td>15.28 (6.16)</td>
<td>16.85 (4.99)</td>
<td>.300</td>
</tr>
<tr>
<td>VAS stress (Mean, SD)*</td>
<td>2.72 (2.59)</td>
<td>3.56 (2.13)</td>
<td>.075</td>
<td>2.66 (2.85)</td>
<td>3.47 (2.22)</td>
<td>.117</td>
</tr>
<tr>
<td>VAS-Visual Analogue Scale</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
* indicates non parametric test
Table 11: Difference in TEWL data between control and intervention groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (all participants; N = 36)</th>
<th>Intervention (all participants; N = 36)</th>
<th>$p$ value</th>
<th>Control (included in TEWL analysis; N = 29)</th>
<th>Intervention (included in TEWL analysis; N = 27)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline TEWL reading, selected disrupted sites (g/h/m²) ($Mean, SD$)</td>
<td>13.71 (2.58)</td>
<td>14.60 (2.06)</td>
<td>.142</td>
<td>13.74 (2.56)</td>
<td>14.61 (2.17)</td>
<td>.176</td>
</tr>
<tr>
<td>Baseline TEWL, control site ($Mean, SD$)</td>
<td>16.04 (3.00)</td>
<td>15.96 (2.15)</td>
<td>.916</td>
<td>16.55 (4.02)</td>
<td>16.15 (2.51)</td>
<td>.656</td>
</tr>
<tr>
<td>TEWL reading after tape stripping, selected disrupted sites (g/h/m²) ($Mean, SD$)*</td>
<td>59.15 (19.69)</td>
<td>52.61 (18.05)</td>
<td>.156</td>
<td>54.29 (19.49)</td>
<td>46.89 (17.57)</td>
<td>.114</td>
</tr>
<tr>
<td>TEWL reading at follow-up, selected disrupted sites (g/h/m²) ($Mean, SD$)*</td>
<td>42.75 (17.67)</td>
<td>40.42 (17.19)</td>
<td>.171</td>
<td>44.96 (17.21)</td>
<td>39.64 (16.03)</td>
<td>.171</td>
</tr>
<tr>
<td>Number of strips ($Mean, SD$)*</td>
<td>10.63 (2.46)</td>
<td>10.65 (2.50)</td>
<td>.972</td>
<td>10.34 (1.86)</td>
<td>10.74 (2.67)</td>
<td>.515</td>
</tr>
</tbody>
</table>

* indicates non parametric test
**Social closeness**

Eighteen dyads completed the relationship induction task to facilitate social closeness. Of the 6 questions in the first list 97% of participants completed at least 5 questions. The second list contained 12 questions and 78% of people completed at least 5 questions. The third list contained 10 questions and 83% of people completed at least 6 questions. On average participants completed 21 questions of a possible 28 (SD = 4). The majority of participants reported they felt they had adequate privacy (94%), they felt comfortable (100%), it was a good way to get to know another person (100%) and they considered conversation an important way of getting to know other people (94%). Only 31% of people said that their friends asked them similar questions and 50% said they often engage in similar conversations. On average participants felt moderately close to their partner (Mean = 4.88, SD = 1.68), moderately similar to their partner (Mean = 5.06, SD = 2.01), liked their partner (Mean = 6.97, SD = 1.35) and felt they could be friends in the future (Mean = 6.23, SD = 1.85).

**Effects of social closeness on skin barrier recovery**

Table 11 shows the TEWL readings after tape stripping and at the recovery point in the 56 participants included in the final analysis. Average skin barrier recovery was significantly lower in the control group (Mean = 23.0%, SD = 7.5%) than in the social closeness group (Mean = 30.2%, SD = 11.1%; t(54) = 2.86, p = .006, r = .36). Several variables that have been associated with skin barrier recovery in other research were significantly correlated with skin barrier recovery in this study: age (r = -.28, p = .038), number of strips (r = -.37, p = .005), and level of skin impairment (r = -.61, p < .001). When these variables were included in the model the differences between groups remained significant (F(1,51) = 4.40, p = .041, partial $\eta^2 = .08$). As baseline VAS stress was almost significantly different between groups this was added as a co-variate in a separate model. The results showed that the difference in skin barrier recovery between groups was still significant (F(2, 53) = 8.30, p = .006, partial $\eta^2 = .14$), and the co-variate VAS stress was not significantly related to skin barrier recovery in this model, F(1, 53) = .26, p = .612, partial $\eta^2 = .01$).
A median split was conducted based on the question asking how much each person liked their partner (Median = 7). Participants who liked their partner more had better skin barrier recovery (Mean = 34.8%, SD = 9.0%) compared to those that rated they liked their partner less (Mean = 26.0%, SD = 11.5%), and this finding was significant, \( t(25) = 2.21, p = .037, r = .39 \).

**Effects of social closeness on self-reported stress**

Figure 13 shows the changes in self-rated stress on the VAS over all four time points. A repeated measures ANOVA with a Greenhouse-Geisser correction showed there was a significant main effect of time, \( F(2.05, 143.63) = 46.77, p < .001 \), partial \( \eta^2 = .40 \), indicating that over time stress ratings significantly decreased in the combined sample. There was also a significant interaction effect \( F(2.05, 143.63) = 3.58, p = .029 \), partial \( \eta^2 = .05 \). Post hoc analyses adjusted for Bonferroni corrections indicated that there was a significant decrease in stress in the social closeness group from before tape stripping to recovery \( (p = .006) \), whereas this was not significant for the control group \( (p = .948) \). Both groups showed significant reductions in self-reported stress from baseline to before tape stripping \( (p < .001) \).

Because baseline VAS stress was almost significantly different between groups, this was added as a co-variate in a separate model. An ANCOVA controlling for baseline stress found that there was a significant difference between groups in change in stress over the study period (from baseline to follow-up). Participants in the intervention group had larger decreases in stress (\( Adjusted mean = -2.59, SE = 0.22 \)) compared to the control group (\( Adjusted mean = -1.90, SE = 0.22 \)), \( F(1, 69) = 4.79, p = .032 \), partial \( \eta^2 = .07 \).
Moderation effects on skin barrier recovery

A moderation analysis was performed to see if the amount of change in self-reported VAS stress from after tape stripping to follow-up moderated the effects of social support on healing. This period was chosen because this is the time over which participants were recovering. The results showed a significant interaction effect indicating that the relationship between skin barrier recovery and group allocation was moderated by changes in stress, $b = 5.65$, 95% CI [0.42, 10.88], $t = 2.17$, $p = .035$. Simple slopes analysis showed (1) there was a significant relationship between performing the intervention and better skin barrier recovery when there were large reductions in stress, $b = -11.63$, 95% CI [-17.80, -4.82], $t = -3.50$, $p > .001$, (2) there was a significant relationship between performing the intervention and better skin barrier recovery at the mean value of stress reduction, $b = -7.19$, 95% CI [-12.38, -2.00], $t = -2.78$, $p = .008$, (3) there was a non-significant relationship between performing the intervention and skin barrier recovery for the lowest level of stress reduction, $b = -
3.07, 95% CI [-0.96, -2.00], t = -2.78, p = .400). This suggests that the intervention improved healing only in people who reported at least moderate stress reduction over the recovery period.

**Salivary cortisol**

A repeated measures ANOVA with a Greenhouse-Geisser correction was conducted with the transformed data set to investigate differences between groups over time in cortisol. There was a main effect of time $F(1.96, 137.07) = 23.45$, $p < .001$, partial $\eta^2 = .25$, indicating that cortisol decreased over the study time period for both groups. There was not a significant interaction effect, $F(1.96, 137.07) = 1.30$, $p = .276$, partial $\eta^2 = .02$. Change scores over the reactivity period (from baseline to after tape stripping) showed there were no significant differences between groups when controlling for baseline, $F(1, 69) = .40$, $p = .530$, partial $\eta^2 = .01$. Similarly, there were no significant differences in change scores between groups over the recovery period, $F(1, 70) = .86$, $p = .356$, partial $\eta^2 = .01$. Figure 14a shows the raw data before Log10 transformation was applied.

The total cortisol response over the time period calculated by formulating area under the curve with respect to increase (AUC) was not significantly different between the social closeness and the control group, $U = 556.00$, $z = -1.04$, $p = .300$.

**Salivary alpha-amylase**

A repeated measures ANOVA with a Greenhouse-Geisser correction was conducted with the transformed data set to look at differences in alpha-amylase between groups over time. There was a main effect of time $F(3, 201) = 6.60$, $p < .001$, partial $\eta^2 = .09$, indicating that alpha-amylase increased over the study time period in the combined sample. There was not a significant interaction effect between groups over the four time periods although this approached statistical significance, $F(3, 201) = 2.24$, $p = .084$, partial $\eta^2 = .03$. Change scores over the reactivity period (from baseline to after tape stripping) showed there was a significant difference between groups when controlling for baseline, $F(2, 66) = 6.90$, $p = .011$, partial $\eta^2 = .20$. The control group had a significantly greater increase ($Mean\, increase = 35.25U/ml, SD = 84.31$) than the intervention group ($Mean\, increase = 18.31U/ml, SD = 79.78$). There were no differences in change scores between groups over the
recovery period, $U = 495.00$, $z = -1.55$, $p = .121$. Figure 14b shows the raw data before Log10 transformations were applied.

Total alpha-amylase response over the time period calculated by formulating area under the curve with respect to increase (AUC) was not significantly different between the social closeness and the control groups, $U = 569.00$, $z = -.51$, $p = .613$. 
Figure 13. Salivary cortisol (a) and alpha-amylase (b) over time between the social closeness and control groups.
Discussion

This is the first study to show that undergoing a tape stripping procedure in the presence of a peer with a close relationship can improve skin barrier recovery compared to undergoing tape stripping alone. Skin barrier recovery was significantly better in the intervention group, even after controlling for several known variables that were correlated with skin barrier recovery. The social closeness intervention reduced self-reported stress over the study period, and dampened alpha-amylase reactivity. Participants who better liked the person they were partnered with during the study had better healing than those who liked their partner less, which suggests the nature of the relationship formed was important. Furthermore, the effects of the intervention were strongest for those people who experienced a larger reduction in stress over the recovery period.

The improvements in healing in the intervention group may operate via autonomic nervous system reactivity, as indicated by a lower salivary alpha-amylase response. However, the difference in cortisol between groups was not significant. Potentially, the intervention directly reduced stress or served as a distraction, reducing autonomic nervous system arousal. This finding supports previous research that showed social housing buffered the effects of restraint stress on hamsters’ wound healing via endocrinological pathways (Detillion, et al., 2004). However, more research needs to be conducted to confirm that social closeness can promote wound healing through stress reduction pathways.

This research provides further evidence that the presence of a peer with a close social relationship may reduce autonomic arousal, without the need for active social support. Overall these findings are in line with animal studies showing that mice have better healing if they are housed with a partner (Glasper & DeVries, 2005) and experimental studies with humans that show that people have faster healing if they have a supportive conversation with their partner (Kiecolt-Glaser et al., 2005). This finding also supports Edwards et al., (2009) finding that a peer support group for patients with leg ulcers can improve healing. Our findings may differ from the null findings in the study on the
provision of social support from a confederate for several reasons (Robles, 2007). First, we conducted a relationship closeness induction task so the participants felt close to each other prior to tape stripping, rather than having an unfamiliar confederate present. Second, participants provided passive support, rather than a confederate providing active support. And third, the participants were facing the same stressor rather than having different roles.

This research had some limitations. First, it was conducted in a laboratory setting and may not be generalizable to a hospital setting. Second, the research used a contrived social interaction task. The forced interaction may have been uncomfortable for some participants and a genuine social interaction may be hard to reproduce in everyday life. Third, the study had a small sample size and future research should replicate these results with larger samples. A fourth limitation was that the researcher performing the tape stripping could not be blinded to the condition of each participant because those in the intervention group had the procedure performed while another participant was in the room.

A final limitation was that the sample in this study consisted of young healthy participants and this makes it difficult to directly apply the findings to a clinical population. Tape stripping creates only a minor skin abrasion, associated with little pain, and healing occurs over a short time frame. The healing of this skin wound may follow a different trajectory to those in a clinical setting, who have undergone surgery or have chronic ulcerations, and may have other co-morbidities that influence healing time. More research needs to be conducted in clinical settings.

While patient support groups are popular in community settings, support from fellow patients in hospital is usually ad-hoc and further research is needed to develop and test supportive interventions in this area. It may be helpful to offer hospital patients the chance to talk with others undergoing the same procedure, either beforehand, afterwards, or both. This is facilitated when patients waiting for a procedure are located in beds next to patients who are recovering from the same procedure. This offers the chance for patients to see and hear other patients in similar
circumstances, which may help anxious patients feel less alone and also provide the opportunity for social modelling to occur (Symbaluk, Heth, Cameron & Pierce, 1997).

**Conclusions**

These preliminary findings suggest that undergoing a similar procedure with someone with whom you have established a short-term close relationship can reduce self-reported stress, reduce activation of the autonomic nervous system as indexed by alpha-amylase, and positively influence healing. Future research could investigate whether interventions can be designed to systematically develop closer relationships between patients in hospital settings to improve wound healing following medical procedures.
Chapter 9: Discussion

Research in the field of psychoneuroimmunology has demonstrated that psychological stress and other psychosocial variables can alter immune function and negatively influence wound healing. In light of this research, more importance has been placed on the potential for psychological interventions to improve immunological and healing outcomes. The aims of the work presented in this thesis were to systematically review the research conducted on the effects of psychological interventions on wound healing, identify areas where there was a need for more research; and to further investigate whether psychological interventions could improve healing. The research conducted in this thesis shows that further research in clinical populations should focus on exploring the efficacy of different types of psychological interventions and when they should be implemented with surgical patients or with those who have chronic wounds.

In this discussion the key findings from this thesis are summarized. Then the results are discussed in light of other research that has used similar psychological interventions. The contributions of this thesis to existing knowledge are highlighted and possible implications of the research for clinical practice are described. Lastly the limitations and areas for future research are discussed, in terms of how these limitations can be addressed in future studies and the need for more clinical trials measuring psychosocial interventions and wound healing.

Summary of key findings

The research presented in this thesis has several key findings. First the systematic review (Robinson, Norton, Jarrett & Broadbent, in press) in Chapter 3 summarised previous research investigating the impact of psychological interventions on wound healing. The rationale for conducting this review stemmed from robust findings showing that stress and other psychological factors can slow wound healing. Indeed the meta-analysis by Walburn et al. (2009) concluded by noting that more experimental research was needed looking at how reducing psychological stress can help determine the causal relationship between psychological factors and healing. Furthermore,
the authors of this review wrote that research should be conducted in healthy populations with experimental wounds before such interventions could justifiably be introduced in clinical settings. Hence, a systematic review was the first step in understanding where the current literature stood regarding psychological interventions and wound healing. A systematic review focusing on interventions looking at wound healing has not been conducted previously and the review synthesized the existing literature. It provided a critical starting point for conducting further research in this area, based on the identified gaps in the literature.

The review demonstrated that a number of psychological interventions have been trialled in both clinical and experimental settings, many of which showed favourable effects on wound healing. However, the review also showed that there was a great amount of variation in the type of wounds assessed (chronic wounds, surgical wounds, fracture, burns, and experimental wounds), the ways the wounds were assessed, the type of interventions trialled, and the time period over which the interventions were implemented. The interventions identified in the review were relaxation, social support, expressive writing, yoga, hypnosis, placebo, distraction, stress management, empathetic care and cognitive behavioural therapy. The most support was shown for relaxation and expressive writing interventions, although there were insufficient studies and/or too much variation between studies to conduct a meta-analysis. Three interventions had been conducted looking at social support in various contexts and although the results from these studies were not significant, they appeared promising, warranting further research. Therefore these three types of intervention were targeted in this thesis. Furthermore, analysis of the variation in the studies provided structure to guide this research in terms of methods.

One important issue raised from the review was whether the timing of the intervention (either before or after wounding) made a difference to the effectiveness of the intervention. Due to the variation in settings and the type of wounds, some interventions in the review were administered before wounding, some were administered after wounding and others spanned the pre and post wounding period. Timing was identified as important for both practical reasons (for
example, identifying whether interventions should be delivered before or after surgery), as well as theoretical reasons (identifying the mechanisms of how the intervention improve healing). This question of timing was subsequently explored in studies 1 and 2 of this thesis.

A second important issue identified in the systematic review was that interventions focusing on social support varied in the way they were implemented. One intervention was conducted with a peer support group, one intervention aimed to buffer stress related to a performance task, while the last intervention was conducted prior to surgery with patients. Therefore, more research on social support interventions was identified as a key area for future work. This was explored in the last study conducted as part of this thesis.

Study 1 investigated the timing of a relaxation intervention on healing, which appears in Chapter 4 of this thesis. (The systematic review included Study 1 (Robinson et al., 2015), because Study 1 was published before the review was submitted for publication). Study 1 randomised participants to one of two intervention conditions where the relaxation was performed either immediately before or immediately after the tape stripping procedure. The results showed that regardless of when participants performed the relaxation intervention, they showed improved healing indicated by faster skin barrier recovery. Participants in the intervention conditions also reported that they were significantly more relaxed and experienced significantly less pain than those in the control group. These findings suggest that relaxation is warranted both before and after wounding, and may help to not only reduce stress but also reduce pain. Subsidiary findings from Study 1 appear in Chapter 5 as a separate publication (Robinson, Jarrett & Broadbent, in submission). The key finding from this chapter, confirms previous research showing that sleep is a critical factor in wound healing, and should be assessed. In this chapter, participants were divided based on sleep on the previous 24 hours. The findings showed that more sleep was related to faster skin barrier recovery and lower stress levels, indicating that stress, sleep and skin barrier recovery are intertwined.
The second experimental study of this thesis (Study 2; Robinson, Jarrett, Vedhara & Broadbent, 2017), again built on the findings from the systematic review, identifying the need for more research within each intervention category. In this case expressive writing was investigated, specifically focusing on the timing of the intervention to determine how expressive writing impacted the healing process. This study randomised participants to a control or intervention condition in a 2 x 2 design based on the timing of the intervention. The study was conducted with healthy participants, and investigated the healing of a 4mm punch biopsy wound to the upper arm 10 and 14 days after wounding, by ratings of re-epithelialisation. The major findings presented in Chapter 6, were that the timing of an expressive writing intervention was critical to re-epithelialisation. Those assigned to write expressively prior to the biopsy had better healing in comparison to both control groups. Furthermore, the data suggested that expressive writing causes a temporary negative change in affect, which may explain why those assigned to write expressively after the biopsy did not show any improvements in healing in comparison to the other groups. To illustrate the importance of timing, within the expressive writing group who wrote after the biopsy, those who completed the writing task quickly had better healing than those that did not after 2 weeks later. No previous studies have investigated the impact of performing expressive writing after wounding on healing.

Chapter 7 (Robinson, Jarrett, Vedhara, Tarlton, Whiting & Broadbent, in preparation) was a continuation of Study 2, and described the immunohistochemical analysis of the healing skin. A second biopsy was taken of the wound at 14 days to analyse cells in the healing tissue. No previous studies have used this method of taking a biopsy of a healing wound for analysis. The key findings from this study were that those that performed expressive writing (either before or after wounding) had better healing than the control groups based on the Langerhans cell counts. Furthermore, Langerhans cell activation was significantly associated with lower levels of neutrophils and macrophages. During the inflammation phase of healing neutrophils and macrophages should be abundant in the wound (Landén et al., 2016). Low levels of neutrophils and macrophages and high levels of Langerhans cells at 14 days indicate that the wound has moved to the proliferation phase of
healing (Meszaros et al., 2000). This study provides valuable insight into how the expressive writing intervention affects healing on a cellular level. It is also the first study to show that expressive writing both before and after wounding can have beneficial effects on Langerhans cells in healing tissue.

The final experimental study was presented in Chapter 8 (Study 3; Robinson, Ravikulan, Nater, Skoluda, Jarrett & Broadbent, 2017). This study investigated how social support provided through interactions between participants undergoing a wounding procedure may help promote wound healing. All participants underwent a tape stripping procedure but were randomly assigned either to a control condition (where they were alone) or to an intervention condition (where they were with another person they had not met before). Prior to the session, participants in the intervention group performed a brief task to help foster social closeness, they then underwent the tape stripping procedure together and were able to interact during the 30 minute recovery period. The results indicated that those in the social closeness condition had larger reductions in self-reported stress and faster skin barrier recovery. Within the intervention group, participants who said they liked their partner more had the best recovery. In this study saliva samples were taken to see if the social closeness intervention helped to buffer stress reactions. The findings showed that those in the social closeness condition had lower autonomic nervous system reactivity, suggesting the intervention had a stress-buffering effect.

Overall, these three experimental studies contribute to the existing research on wound healing by providing further evidence that relaxation, expressive writing and social support interventions can improve healing.

**Integration into current literature**

The findings from the experimental studies in this thesis add further support to the evidence described in the initial systematic review, which showed that psychological interventions are beneficial for wound healing. In this section, each study will be briefly discussed in terms of how the findings compare to previous research.
Relaxation

Relaxation is widely known to affect a number of health outcomes (Gruzelier, 2002; Luebbert et al., 2001; van Dixhoorn & White, 2005) and to reduce stress and anxiety (Bastani, Hidarnia, Kazemnejad, Vafaei & Kashanian, 2005; Manzoni, Pagnini, Castelnuovo & Molinari, 2008; Smith et al., 2007). The findings from Study 1 support this research by showing that relaxation can improve skin barrier recovery (Robinson et al., 2015). Previous research indicates that relaxation prior to surgery is beneficial in reducing haematomas, pain and analgesic consumption after inguinal hernia repair (Omlor, Kiewitz, Pietschmann & Roesler, 1999) and can improve activity levels and pain in cholecystectomy patients (Miró & Raich, 1999). Research also suggests that relaxation post-surgery can significantly reduce pain levels (Good et al., 2005; Topcu & Findik, 2012), stress and anxiety (Choi & Eun, 2010). However, no research has specifically addressed when it is preferable to perform relaxation and how it may relate to wound healing outcomes. Previous research looking at wound healing after surgery has implemented relaxation before surgery that was continued after surgery (Broadbent et al., 2012; Holden-Lund, 1988).

As research has shown that relaxation has a positive effect on healing, research has focused on the mechanisms via which this may affect the immune system. It is thought that the relaxation response counteracts the physiological arousal caused in reaction to stress. For example, research has found that relaxation lowers cortisol levels (Pawlow & Jones, 2002) implying immunoenhancement, and reductions are greater with more frequent practise (Cruess et al., 2000). Research indicates that use of relaxation techniques can improve cell-mediated immunity over time (McGrady, et al., 1992; Zachariae et al., 1990). In Study 1, cortisol and immune parameters were not measured. However, participants did report feeling more relaxed than the control group.

Study 1 also found that participants who performed relaxation reported less pain. This finding shows that it is also important to consider the impact relaxation may have on other variables that are related to wound healing. Relaxation leads to a reduction in the sympathetic response to
pain by decreasing blood pressure, heart rate, respiration and muscle tension as well as decreasing the cognitive awareness of pain (Friesner, Curry & Moddeman, 2006). As pain is known to slow wound healing (McGuire et al., 2006), part of the benefits of relaxation might be attributed to reductions in pain. This fits with previous research reporting that relaxation can help reduce pain after surgery (Good, 1996) and in experimental settings (Busch et al., 2012).

Sleep

A subsidiary finding from Study 1, presented in Chapter 5 (Robinson, Jarrett & Broadbent, in submission) showed that when participants were assessed based on the amount of sleep they had over the past 24 hours, there were differences in skin barrier recovery. Those who reported more sleep, had faster healing. This finding is in line with other research that has looked at skin barrier recovery and sleep in women finding that skin barrier recovery is impaired after sleep deprivation (Altemus et al., 2001) or smaller amounts of sleep (Benham, 2015; Oyetakin-White et al., 2015). Furthermore, research indicates that sleep is important for wound healing (Koschwanez et al., 2013). This study highlights that sleep is important to consider as another variable that may influence healing. Sleep is important for immune functioning (Lange et al., 2003; Ruiz et al., 2007) and consequently may impair healing after surgery, especially given that sleep disturbances are common post-surgery due to pain and noise (Closs, 1992; Rosenberg, 2001).

By investigating the association with sleep and skin barrier function in a healthy population, the findings presented in this thesis highlight the importance of sleep for healthy skin function. Research has shown that lack of sleep can have an impact on autoimmune conditions, including dermatological diseases (Koo, 1996; Strober et al., 2012). This is important, considering conditions such as atopic dermatitis and psoriasis are already characterised by impairments in skin barrier function (Gupta & Gupta, 2013) and sleep restriction is associated with increases in inflammation (Mullington, Simpson, Meier-Ewert & Haack, 2010).
Expressive writing

Expressive writing, like relaxation, has been the focus of a great amount of research, which has detailed the positive effects of writing on a number of health outcome outcomes (Frattaroli, 2006). However, few studies have been conducted looking at the impact of expressive writing on wound healing. The results from Study 2 confirm the findings from the other two studies that have been conducted using similar methods (Koschwanez et al., 2013; Weinman et al., 2008). These two studies found that performing expressive writing prior to wounding promotes wound healing. This research builds on these two research studies, one of which notes (Weinman et al., 2008) that future research should investigate whether expressive writing could be effective post-biopsy. In Study 2 participants were allocated to write after the biopsy was performed. The rationale for exploring the impact for timing was to determine how such an intervention could realistically be implemented in clinical care.

In Study 2 participants allocated to write after wounding showed improved healing if they completed the writing task within a short time frame, starting immediately after wounding, relative to others in the same group, who delayed starting the writing task. This finding offers some interesting insights, given that no other studies have conducted the writing intervention immediately after wounding. In contrast, other research has focused on long-term benefits of expressive writing in patients with chronic illnesses (Kelley et al., 1997; Vedhara et al., 2007) To illustrate this focus on long-term benefits, in a meta-analysis investigating moderating variables, Frattaroli (2006) reported that of 146 studies included in the analysis 23% had follow-ups less than a month after expressive writing, with the average follow-up point being 3 months later. Other meta-analyses (Frisina, Borod & Lepore, 2004; Smyth, 1998) excluded studies with follow-up periods of less than 1 month because of concerns of the impact of short-term negative effects of writing. Hence, it appears that expressive writing benefits occur over time but it is not known how expressive writing can effect healing over a short period of time. The current research suggests that with acute wounds, the sooner expressive writing is finished, the sooner benefits can occur.
Study 2 adds to research showing that expressive writing can cause a short-term increase in distress (Booth et al., 1997). These changes in affect may be linked to sympathetic activity and release of stress hormones (Miller & Cohen, 2001; Petrie et al., 1995). The findings of Study 2 illustrate that expressive writing caused changes in affect, which may be related to changes in healing. A temporary increase in positive affect and decrease in negative affect during healing may be associated with better healing. This may be important because other research has found that positive affect is related to faster skin barrier recovery (Robles et al., 2009) and positive and negative affect is linked to changes in cortisol levels (Herbert & Cohen, 1993; Steptoe et al., 2008), which may influence healing.

The findings from this research indicate the expressive writing should be trialled in clinical settings. Very few studies have assessed wound healing in clinical care as part of an expressive writing intervention. One study conducted by Solano and colleagues (2003), asked participants hospitalised for bladder papilloma resection to complete an expressive writing intervention around their thoughts about their upcoming operation. Participants started writing between 3 and 4 days before their operation and wrote for 20 minutes a day over three days. Compared to a control group, participants assigned to expressive writing had significantly shorter hospital stays and fewer physical symptoms, but the study did not assess healing itself.

The immunohistochemical analyses of the wound tissue from the healing biopsies support the findings from Study 2 showing that expressive writing prior to wounding can improve wound healing. However, since the analysis is not yet completed only the preliminary results can be discussed. The research found that participants who performed expressive writing had higher levels of Langerhans cells compared to the control groups. In the sample, lower levels of macrophages and neutrophils were negatively associated with Langerhans cells as well as whether or not participants were healed ten days after the biopsy. This suggests that immunological changes within the wound can influence healing and that the expressive writing intervention influenced these immune processes. This fits with other research that has noted the effect of stress on immunohistochemical
outcomes. For example, Koschwanez et al., (2015) reported that in skin sample removed during a punch biopsy study, lower activation of immune cells in the skin and fewer Langerhans cells were associated with slower healing. Other research also supports the role of Langerhans cells in faster wound healing in diabetic ulcers (Stojadinovic et al., 2013). Finally, in another study (Kleyn et al., 2008), participants had a biopsy taken and were then subjected to a stress task. They then had another biopsy taken from the same area after the stress task. Compared to participants that did not perform the stress task, participants who were stressed had a significant reduction in epidermal Langerhans cells from baseline to follow-up, whereas there were no significant changes in controls. This suggests that stress can modulate the cutaneous immune responses through the regulatory role of Langerhans cells in immunity (Ruiz, Quinones, Diaz & Tapia, 2003). At present this is the only study to look at immunohistochemical markers in healing tissue after a psychological intervention and provides initial indications of the mechanisms involved in promoting healing.

**Social support**

The results of the social closeness intervention are supportive of the general notion that social support can help promote better health outcomes. Study 3 took a novel approach to investigate the affect of pairing participants with one another for a tape stripping procedure. Previous research investigating wound healing has looked at social support in various different ways, but only one study looked at the impact of social support from peers with similar health problems (Edwards et al., 2009). In a study with people with chronic leg ulcers, people in a support group had better wound healing, compared to a control group in terms of ulcer size, although not in percentage reduction in ulcer area. They also had high self-esteem, better quality of life, less pain and more functional ability than the control group. However, this study did not measure stress or look at any look at changes in neuroendocrine markers, which may impact wound healing.

The current findings fit with the idea that social support from people in similar situations can reduce stress and positively influence heath outcomes post-surgery (Kulik & Mahler, 1989; Thoitis et
For example, research has found that being put in a room with another person who has undergone a similar cardiac procedure can help reduce anxiety (Kulik et al., 1996). Furthermore, patients assigned to room with another cardiac patient, regardless of whether they were pre or post operation, had shorter stays in hospital compared to people in a room by themselves or with a patient that was undergoing a different procedure. The authors hypothesized that being paired with another person allows for emotional support based on their similarities. This research draws on a theory proposed by Schachter (1959) who suggested that when people are facing a novel threat, they will experience an increased desire to affiliate with others who are facing the same threat and are of a similar emotional status. For example, research conducted found that when people were made to wait to receive an electric shock, they preferred to wait with someone who was also facing a similar threat and acting in a similar way to the prospect of the shock (Darley, 1966; Darley & Aronson, 1966; Firestone, Kaplan & Russell, 1973). Hence people are able to validate their feelings based on what another person in the same situation is feeling and are motivated to interact to gain support and reassurance, resulting in less anxiety. Research has also been conducted with students who were asked to imagine two scenarios (a diagnosis of cancer, and a bad grade on a test) and to rate how they would react to a person in a similar situation to them, a worse situation to them or a better situation to them (Helgeson & Mickelson, 1995). The results showed that participants reported they were motivated to associate with people in a situation similar to themselves because they had a common bond (a need for empathy and support, to not feel alone and isolated, a shared experience and things in common). This shows that being paired with a stranger in a similar and uncertain situation, as in Study 3, is a form of social support that people seek and may provide benefits. This supports other research that shows that bonding with a similar other can enhance mood, compared to bonding with people who are better or worse off (Helgeson & Taylor, 1993; Wheeler & Miyake, 1992). Having a common bond with a similar others may also explain motivation to join support groups (Taylor, Falke, Mazel, Hilsberg & Gottlieb, 1988) and indicates that peer support may be underused but potentially beneficial intervention for patients (Colella & King, 2004).
The findings from Study 3, support these findings, particularly the results showing that participants who liked one another more (had a stronger emotional bond or felt more social closeness) had better skin barrier recovery.

Study 3 builds on previous research by showing that support from another person can help reduce self-reported stress and can reduce autonomic activity associated with stress. Previous research has found that the physiological changes that occur during supportive interactions can buffer autonomic nervous system activation and reduce cortisol release through HPA axis (Heinrichs et al., 2003). The current research supports this because participants in the control group had a significantly greater increase in alpha-amylase over the tape stripping period indicating changes in the autonomic nervous system. Social support in its various forms is thought to protect individuals from stress by offering them more coping strategies (Lazarus & Folkman, 1984). This research fits with the stress-buffering theory that psychological interventions can reduce or buffer the effects of stress, as they modulate the same physiological pathways of the neuroendocrine system. This is in support of previous research that has found that social support buffers the effects of stress through endocrinological pathways (Detillion, et al., 2004). However, in light of these findings more research is needed to understand how support from another person in the same situation can reduce stress and subsequently influence on healing.

**Clinical implications**

Acute surgical wounds as well as chronic wounds are at risk of infection and complications further placing demands of healthcare resources. The economic and social impact of wound care is of great importance, and research needs to be conducted to understand the factors that influence wound healing and recovery (Sen et al., 2009). The collection of experimental studies presented in this thesis offer insight into how psychological interventions may be useful in clinical settings.

As previously noted, surgical procedures represent a huge stressor for any patient. Surgery is associated with a great deal of anxiety (McCleane & Cooper, 1990) and the recovery period can
often be uncomfortable and painful. Research shows that a number of psychological factors, particularly mood, distress and coping styles can negatively influence surgical outcomes, including length of stay anaesthesia requirements, complications, immune function and functional recovery (Rosenberger et al, 2006; Stengrevics et al., 1996; Vollmer-Conna, et al., 2009). These findings highlight how psychological factors can have clinically relevant outcomes, and should be addressed to promote better recovery.

Research investigating the impact of psychological interventions on wound healing demonstrates that psychological factors, neuroendocrine responses and immune function are all interrelated and this has important implications for clinical care. Specifically, this research demonstrates that three psychological interventions, (relaxation, expressive writing and social bonding) can have an impact of wound healing and associated outcomes related to recovery. Although this research has not been conducted in clinical settings, the findings indicate that trials should be conducted with patients to understand how these findings translate into practice. This thesis addressed how the timing of such interventions may impact wound healing and the understanding of when to intervene is a logistical question that is important to address if such interventions are to be used in clinical settings.

These studies also have implications for dermatological disease, particularly research conducted using the tape stripping model. Research shows that stress impairs skin barrier recovery (Garg et al., 2001, Robles, 2007) and can exacerbate skin diseases associated with abnormal cutaneous inflammatory responses such as psoriasis and atopic dermatitis (Al’Abadie et al., 1994, Locala, 2009; Tausk & Nousari, 2001). For example, research has found that psoriasis patients receiving photochemotherapy treatment for their disease take longer to clear psoriatic lesions if they report higher levels of stress and anxiety (Fortune et al., 2003). Furthermore, patients that report low levels of social support have greater exacerbations of diffuse plaque psoriasis (Picardi et al., 2005). Hence, the findings of this thesis may have relevance for patients with skin disease, again
highlighting how psychological interventions may be useful in clinical practice. Research has found that patients with psoriasis and atopic dermatitis show improvement following relaxation interventions (Kabat-Zinn et al., 1998; Keinan et al., 1995; Shenefelt, 2010) and cognitive behavioural interventions to reduce disease-related stress (Fortune, Richards, Griffiths & Main, 2004). Psychological interventions for this patient group should be considered given the findings of studies 1 and 3, which were both conducted using the tape stripping model and show the alterations in the process of skin repair relevant to skin disease. In particular, Study 1 shows that relaxation can improve skin function and Study 3 shows that reductions in self-rated stress as well as autonomic arousal may influence skin healing. Furthermore, Study 3 shows that it is important to measure self-rated stress and physiological measures of stress in patients with skin conditions to understand how reductions in perceived stress translate to improvements in disease state.

**Limitations and areas for future research**

Overall, the studies presented in this thesis add to the existing body of experimental studies investigating the impact of psychological interventions on wound healing. This research has used novel methods to explore the question of timing of these interventions in order to understand when they should be implemented. Furthermore, this research has explored the potential mechanism for these effects by including physiological measures and assessing immune markers in healing tissue samples. However, this work is not without limitations. Given the scope of the research undertaken as well as constraints on budget and time, there are many future areas to address and that warrant further research. These are described in the next section in greater detail.

One of the largest limitations of this research is that all of the experimental studies have been conducted with healthy young participants with standardised wounds. This means that although the findings show that psychological interventions can be of benefit in a controlled experimental setting, they cannot be directly generalised to clinical settings. The exclusion and inclusion criteria mean that the experimental studies presented have high internal validity but at the
expense of generalisability. In healthy participants, there are no co-morbidities or any other complications that need to be taken into account, as there might be in clinical settings. The process of wound healing is complicated and a number of other factors may also influence the outcomes of randomised controlled trials. For example, in these studies the wounds that participants received were, in comparison to surgical wound, reasonably mild and associated with little pain or stress. Furthermore, the participants who self-selected to take part in this study, the majority of whom were medical students, may have been less intimidated by the medical procedure, particularly the biopsy, and hence experienced less stress. In comparison, most people that have to undergo surgery are stressed and do not have the luxury of opting out of the procedure, whereas participants in an experimental study could have. Participants in these studies reported relatively little stress and in Study 2 changes in self-reported stress was not observed, nor was baseline stress associated with healing. This is not to say that changes in stress did not occur as a result of the intervention, but the scale used to assess stress may not be sensitive enough to detect subtle changes. However, in clinical populations the link between stress and healing may become more clear and should be researched. The next step in this research is to apply these findings to clinical settings with patients who are undergoing surgery or have chronic wounds to see if psychological interventions are beneficial.

Another limitation of this body of research was the sample sizes of each study. For each study a power calculation was conducted to determine the sample size. However, due to attrition and exclusion of participant data, the number of people included in some analyses was smaller than originally intended. This limited the power of the studies and future research should aim to replicate these findings in larger samples.

In this thesis a number of measures of psychological factors, immune factors and neuroendocrine factors were obtained throughout the three studies to investigate potential mediators of psychological interventions. However, not all of these three variables were measured in each study. The main aim of the thesis was to demonstrate that psychological interventions can
have a positive impact on wound healing, and so it follows logically after this has been demonstrated to investigate why psychological interventions have an impact on wound healing. In order to do so, measures of neuroendocrine hormones offer an insight into how stress and the reduction of stress can influence wound healing, even if decreases self-reported stress was not observed. This was examined in Study 3, but not in studies 1 or 2. The inclusion of a salivary cortisol and alpha-amylase measure should be included in future research and may help explain the pattern of results presented in studies 1 and 2. Similarly, measures of immune function within the wound were only collected in one study, (Study 2, Chapter 7). This is partially due to the type of wound measured in Study 1 and 3, as tape stripping does not allow for the measurement of cells within the tissue. However, research could consider taking blood samples to measure immune markers within the blood, or conducting research with either biopsy or blister models when wound tissue or fluid can be sampled to look at the mechanism via which the intervention may help wound healing.

Another limitation of these studies is the methods used to assess healing. Although the methods used in this study have been validated and used in previous studies (Robles, 2007; Koschwanez et al., 2013) there are some disadvantages to the methods that should be acknowledged. Previous research using tape stripping procedures have been conducted in temperature and humidity controlled laboratories. Unfortunately, access to this was limited meaning that the room in which the tape stripping was conducted was not as stringently controlled as other studies. As a result, the raw TEWL data in studies 1 and 3, was more variable than in previous research. In Study 2, wound healing was assessed by a qualified dermatologist. However, previous research has demonstrated that high resolution ultrasound scanning of a standard punch biopsy wound is a more valid measure of healing activity in deeper tissue layers than surface photography (Dyson et al., 2003; Ebrecht et al., 2004). This is because the wound diameter measured in a photograph is influenced by variable contractions of the wound eschar. In contrast, ultrasound scanning can measure the base of the biopsy (i.e. at the level of the dermal/hypodermal junction), which gives a better assessment of the wound healing process and the structural changes
within the wound that is not affected by changes at the surface if the wound. Access to this equipment was not available. Future research should consider replicating each of the experimental studies in this thesis with different types of experimentally induced wounds and different methods of assessment.

In this research wound healing was assessed at set time points, and each study had a relatively short follow-up period. Other types of wound or methods of tracking healing may allow for more regular assessments of healing. A specific limitation of Study 2 was that psychological measures were assessed over three intervals that were two weeks apart. Better insight may have been gained from more regular assessments of mood and stress, particularly over the healing period. It would also be interesting to look at immune markers within the tissue earlier in the healing process and more regularly, instead of two weeks later, by which time many inflammatory markers had disappeared. In studies 1 and 3 skin barrier recovery was assessed immediately and consequently the recovery time frame chosen was much shorter than previous studies (Altemus et al., 2001; Muizzuddin et al., 2003), although shorter time frames have been reported in previous research (Benham, 2015). The reason for this was also partially due to limitations on participants’ time and limits to the financial incentive offered. However, a limitation of these studies is that skin barrier recovery over a longer period of time was not investigated and should be considered in future research.

Overall the findings of this thesis and the collective limitations of each of the studies create more questions about how and when to administer psychological interventions and for whom specific interventions would be most appropriate. By measuring biological variables, this research strengthens the research linking positive psychological states to improved wound healing and provides some insight into how this may occur. There are many more avenues to consider with this research to build on the findings presented in this thesis.
Conclusions

The keys findings from this thesis are that psychological interventions can improve wound healing, specifically relaxation, expressive writing and social support. Furthermore, the timing of the intervention may impact the intervention efficacy. This research found that relaxation either before or after wounding can have a benefit, but the findings related to the timing of expressive writing are more complex. Research shows that the benefits associated with relaxation are related to reductions in physiological arousal. In contrast, the benefits associated with expressive writing take longer to occur and are likely related to the processing of a distressing event. However, the process of expressive writing can be immediately upsetting and benefits are only seen after initial distress has dissipated. It also appears that expressive writing may not operate via reductions in stress, but changes in affect, which may effect healing via the release of neuroendocrine hormones. Social closeness, in contrast, did appear to reduce self-reported stress and autonomic nervous system activation as well as improve skin barrier recovery. Overall, this research adds to the literature exploring the effects of psychological interventions on wound healing in experimental contexts with standardised wounds. The interactions between psychological factors and wound healing are complex and more research is needed on psychological interventions to improve healing in both laboratory based and clinical settings.
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Appendix A: Study 1 Ethics Approval, Participant Information Sheet and Consent Form

UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE

07-Oct-2013

MEMORANDUM TO:
Dr Elizabeth Broadbent
Psychological Medicine

Re: Application for Ethics Approval (Our Ref. 010545)

The Committee considered your application for ethics approval for your project entitled Improving skin barrier recovery through relaxation. When is the best time to intervene?

Ethics approval was given for a period of three years with the following comment(s):

1. Please add "without giving a reason" to the CF bullet point regarding withdrawal.

2. Please add a bullet point to the CF regarding discomfort and possible redness that could be experienced for 24 hours after the treatment.

The expiry date for this approval is 07-Oct-2016.

If the project changes significantly you are required to resubmit a new application to UAHPEC for further consideration.

In order that an up-to-date record can be maintained, you are requested to notify UAHPEC once your project is completed.

The Chair and the members of UAHPEC would be happy to discuss general matters relating to ethics approvals if you wish to do so. Contact should be made through the UAHPEC ethics administrators at humanethics@auckland.ac.nz in the first instance.

All communication with the UAHPEC regarding this application should include this reference number: 010545.

(This is a computer generated letter. No signature required.)

Secretary
University of Auckland Human Participants Ethics Committee

Dear Participant,

You are invited to take part in a study exploring the effect of relaxation on skin healing.

This project is being carried out by Hayley Robinson, a PhD candidate in the Department of Psychological Medicine at the University of Auckland, Dr Paul Jarrett (Consultant dermatologist, Middlemore Hospital) and Dr Elizabeth Broadbent (Department of Psychological Medicine, University of Auckland), who will be supervising the project.

It is important that you read this document carefully so that you can make an informed decision about whether you would like to participate.

**Purpose of the study:** Previous research has found that a relaxation intervention can have a positive effect on health and can help improve wound healing. The aim of the study is to investigate the effects of relaxation on skin recovery after tape stripping in the laboratory.

**Your rights as a participant:** Participation in the study is entirely voluntary. If you choose to participate, you can change your mind at any time without giving a reason and without any negative consequences. You can withdraw from the study at any time and withdraw any data traceable to you until you have finished the study. Whether or not you participate in this study will not affect your relationship with the researchers. You will be given a copy of this document to keep.

**Procedure:** If you chose to participate in this research you will be asked to come to the University of Auckland to complete a session in the laboratory. In this session first you will be asked some information about your demographics and complete measures about your mood, stress in your life as well as a question about current pain, current stress and how relaxed you feel. You will then be randomly allocated to either get relaxation or not. Depending on the condition you will be guided through a 20 minute relaxation session. An audio will be played to you with instructions and music for you to follow during relaxation. It is very important that you follow these directions for the purpose of this study. The researcher will not be in the room while you have your relaxation. If you are not assigned to the relaxation condition you will be provided with some magazines to read in the room for 20 minutes. You will undergo a tape stripping procedure whereby tape is applied to your skin on your forearm in three 1cm diameter areas just below your elbow. This removes the top layer of your skin. This will be repeated a number of times until your skin barrier function reaches a certain point. To ensure the right level is reached a small probe (1cm in diameter) will be pressed gently against your skin for a 2 minutes or less. At the end of the study skin barrier
function will be measured again using the probe to see how much your skin has recovered. You will be asked to complete measures of mood, pain, relaxation and stress throughout the session.

We are recruiting participants who are aged 18 years or over and speak English. Approximately an hour is required to complete the study at the Department of Psychological Medicine.

You will receive a $10 Westfield voucher at the end of the session.

**Risks and discomfrts:** The procedures outlined in this protocol are minimally invasive and have been performed in other research settings. The tape stripping procedure may cause slight discomfort, and redness of the skin but this should disappear within 24 hours. If you have any allergy to cellophane or adhesives you should not participate in this research.

**Data storage:** All data (questionnaires and skin barrier function) will be stored in electronic format by the researcher. Paper questionnaires and consent forms will be stored in a locked filing cabinet in the researcher’s office at the University, and will be kept for a period of six years.

**Confidentiality:** All personal information will remain strictly confidential and no material that could personally identify you will be used in any report on this study. Participant names will only appear on the consent form, which will be coded with a participant identification number so that your identity is kept confidential on all questionnaires and physiological data files. After completion of the study, all confidential data, including computer data files, will be kept for a minimum period of six years to allow for publication and re-analysis, after which time it will be securely and confidentially disposed of. Research publications and presentations from the study will not contain any information that could personally identify you.

**Results:** A summary of the results of this study will be sent to you if you wish. As it takes some time to analyse the results of studies, it may be more than a year after your participation that you receive this information.

**We appreciate the time you have taken to read this invitation. If you have any questions please contact:**

**Hayley Robinson**
PhD student, Department of Psychological Medicine,
The University of Auckland
Private Bag 92019, Auckland 1142
Email: hrob458@aucklanduni.ac.nz
Phone: +64 9 3737599 Ext. 89454

**Alternative contacts:**
Dr Liz Broadbent, Department of Psychological Medicine,
The University of Auckland
Private Bag 92019, Auckland 1142
Email: e.broadbent@auckland.ac.nz
Phone: (09) 3737599 Ext. 86756

**For ethical concerns, contact:**
The Chair of The University of Auckland Human Participants Ethics Committee,
Office of the Vice Chancellor, Research Office,
Alfred Nathan House, The University of Auckland,
Private Bag 92019, Auckland 1142.
Phone: (09) 3737599 Ext. 87830

Head of Department:
Associate Professor Sally Merry, Department of Psychological Medicine, The University of Auckland
Email: s.merry@auckland.ac.nz

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON
07/10/2013 for 3 years, Reference Number 010545
Project title: The effects of relaxation on health and recovery

Names of researchers: Dr Elizabeth Broadbent (supervisor), Dr Paul Jarrett (co-supervisor) and Hayley Robinson (PhD candidate).

Written Consent Form

I have read and understood the Participant Information Sheet, have understood the nature of the research, and know why I have been selected. I have had an opportunity to ask questions and have had them answered to my satisfaction.

Consent forms will be held for six years in accordance to university policy

- I agree to take part in this research.
- I understand that taking part in this research is voluntary (my choice).
- I understand that participation will take approximately an hour.
- I understand that I am able to withdraw from the study at any time without giving a reason, and to withdraw any data traceable to me until I have finished the study.
- I understand that the overall results may be published in a scientific journal but will not include any information that could identify me.
- I am aware that as a result of taking part in this study once the study session is complete I will be given a $10 Westfield voucher
- I understand that the research data will be stored for 6 years after which they will be destroyed.
- I understand that the tape stripping procedure may cause slight discomfort, and redness of the skin but this will disappear within 24 hours.

Name:.......................................................... ..........................................................

Signature:.................................................. Date:.............................

I wish to receive a copy of the results of this research Yes / No

Please provide an email address or mailing address if you wish to receive a summary of the research findings: ..........................................................................................................................

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE
ON 7/10/2013 for 3 years, Reference Number 010545
Appendix B: Study 2 Ethics Approval, Participant Information Sheet and Consent Form

Office of the Vice-Chancellor
Finance, Ethics and Compliance

UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE (UAHPEC)

16-Aug-2014

MEMORANDUM TO:
Dr Elizabeth Broadbent
Psychological Medicine

Re: Application for Ethics Approval (Our Ref. 012556): Approved

The Committee considered your application for ethics approval for your project entitled Emotional writing and wound healing.

We are pleased to inform you that ethics approval is granted for a period of three years.

The expiry date for this approval is 16-Aug-2017.

If the project changes significantly, you are required to submit a new application to UAHPEC for further consideration.

If you have obtained funding other than from UniServices, send a copy of this approval letter to the Research Office, at re-awards@auckland.ac.nz. For UniServices contracts, send a copy of the approval letter to the Contract Manager, UniServices.

In order that an up-to-date record can be maintained, you are requested to notify UAHPEC once your project is completed.

The Chair and the members of UAHPEC would be happy to discuss general matters relating to ethics approvals. If you wish to do so, please contact the UAHPEC Ethics Administrators at mc.ethics@auckland.ac.nz in the first instance.

Please quote reference number: 012556 on all communication with the UAHPEC regarding this application.

(This is a computer generated letter. No signature required.)

UAHPEC Administrators
University of Auckland Human Participants Ethics Committee

cc. Head of Department / School, Psychological Medicine
Miss Hayley Robinson
Dr Paul Jarrett
Participant Information Sheet

Dear Participant

You are invited to take part in a study exploring the effect of an emotional writing intervention on healing.

This project is being carried out by Hayley Robinson, a PhD candidate in the Department of Psychological Medicine at the University of Auckland, Dr Paul Jarrett (Consultant dermatologist, Middlemore Hospital) and Dr Elizabeth Broadbent (Department of Psychological Medicine, University of Auckland), who will be supervising the project.

It is important that you read this document carefully so that you can make an informed decision about whether you would like to participate.

Purpose of the study: Previous research has found that writing can have a positive effect on health and can help improve wound healing. The aim of the study is to investigate the effects of writing on healing after a 4mm punch biopsy wound and whether writing before or after the biopsy makes a difference to healing.

Your rights as a participant: Participation in the study is entirely voluntary. If you choose to participate, you can change your mind at any time without giving a reason and without any negative consequences. You can withdraw from the study at any time and withdraw any data traceable to you until you have finished the study. Whether or not you participate in this study will not affect your relationship with the researchers. You will be given a copy of this document to keep.

Procedure:

Part 1
If you chose to participate in this research you will be contacted by the researcher. You will be asked to complete baseline measures online that will ask you about your health behaviours, sleep, stress level, mood and social support. You will then be randomised to one of four groups. You will be asked to complete a writing task for 20 minutes each day over 3 days. You will either begin this task after completing the baseline questionnaire or after you have the first biopsy.

Part 2
Two weeks later you will be scheduled to come for biopsy procedure and to complete another questionnaire. The biopsy will be conducted by a registered dermatologist (Dr Paul Jarrett). To take the biopsy the dermatologist will mark a 4mm area on your upper inner arm. He will give you a local...
anaesthetic and will take the biopsy using a sterile, disposable biopsy punch. The wound will be dressed and extra dressings will be supplied. The sample will then be transported to the lab for analysis. If you were assigned to start writing after the biopsy you will start the task on this day (30 minutes).

Ten days after the biopsy is taken you will be asked to come in again to photograph the wound.

**Part 3**

Two weeks after the first biopsy you will come back into the clinic for photographic assessments. You will be asked to complete a final questionnaire. A second 5mm biopsy of the wound site will be taken by the same dermatologist to assess healing and immune markers in your skin (30 minutes).

**Eligibility:** We are recruiting participants who are aged 18 years or over and speak English. You must be a non-smoker. You must be able to understand and write in English. If you have a current inflammatory skin disease, chronic illness, immunological-related health problems or taking medication that impacts immune functioning you will not be eligible for this research. If you are allergic to local anaesthetic you will not be able to take part in this research.

A total of three hours will be required for this study.

**You will receive a $40 Westfield voucher at the end of the study (Part 3)**

**Risks and discomforts:** The procedures outlined in this protocol are minimally invasive and have been performed in other research settings. The biopsy procedure may cause slight discomfort when the local anaesthetic is injected using a needle and syringe. There may also be minor discomfort at the wound site as the local anaesthesia wears-off after the procedure. There is a very small risk of infection at the wound site. The wound will be initially covered by a hydrocolloid dressing, which is sterile and provides an impermeable film to protect against bacteria.

It is not uncommon to report feeling somewhat sad following a writing session. Similar to watching a sad movie, these feelings typically subside within a couple of hours. If you become extremely upset about a writing topic, you should simply stop writing or change the topic of your essays. If you are persistently upset by this experience, we will offer you an opportunity to talk with a student health counsellor or GP.

**Compensation**

In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Accident Compensation Act 2001. ACC cover is not automatic, and your case will need to be assessed by ACC according to the provisions of the Accident Compensation Act 2001. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors, such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses, and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators.

If you have any questions about ACC, contact your nearest ACC office or the investigator. You are also advised to check whether participation in this study would affect any indemnity cover you have or are considering, such as medical insurance, life insurance and superannuation.

**Data storage:** All data will be stored in electronic format by the researcher. Consent forms will be stored in a locked filing cabinet in the researcher’s office at the University, and will be kept for a period of six years. The two samples of your skin will be stored at the University of Auckland and sent to the University of Bristol for analysis.
**Confidentiality:** All personal information will remain strictly confidential and no material that could personally identify you will be used in any report on this study. Participant names will only appear on the consent form, which will be coded with a participant identification number so that your identity is kept confidential on all questionnaires, essays and physiological data files. After completion of the study, all confidential data, including computer data files, will be kept for a minimum period of six years to allow for publication and re-analysis, after which time it will be securely and confidentially disposed of. Research publications and presentations from the study will not contain any information that could personally identify you.

**Results:** A summary of the results of this study will be sent to you if you wish. As it takes some time to analyse the results of studies, it may be more than a year after your participation that you receive this information.

We appreciate the time you have taken to read this invitation. If you have any questions please contact:

**Hayley Robinson**  
PhD student, Department of Psychological Medicine,  
The University of Auckland  
Private Bag 92019, Auckland 1142  
Email: hrob458@aucklanduni.ac.nz  
Phone: +64 9 3737599 Ext. 89454

**Alternative contacts:**  
Dr Liz Broadbent, Department of Psychological Medicine,  
The University of Auckland  
Private Bag 92019, Auckland 1142  
Email: e.broadbent@auckland.ac.nz  
Phone: (09) 3737599 Ext. 86756

**For ethical concerns, contact:**  
The Chair of The University of Auckland Human Participants Ethics Committee,  
Office of the Vice Chancellor, Research Office,  
Alfred Nathan House, The University of Auckland,  
Private Bag 92019, Auckland 1142.  
Phone: (09) 3737599 Ext. 87830

**Head of Department:**  
Associate Professor Sally Merry, Department of Psychological Medicine, The University of Auckland  
Email: s.merry@auckland.ac.nz

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON 16/08/2014 for 3 years, Reference Number 012656
**Written Consent Form**

I have read and understood the Participant Information Sheet, have understood the nature of the research, and know why I have been selected. I have had an opportunity to ask questions and have had them answered to my satisfaction.

Consent forms will be held for six years in accordance to university policy.

- I agree to take part in this research.
- I understand that taking part in this research is voluntary (my choice).
- I understand that participation will take a total of three hours
- I understand that I am able to withdraw from the study at any time without giving a reason, and to withdraw any data traceable to me until I have finished the study.
- I understand that the overall results may be published in a scientific journal but will not include any information that could identify me.
- I am aware that as a result of taking part in this study I will be given a $40 Westfield voucher
- I understand that the research data will be stored for 6 years after which they will be destroyed.
- I understand that the biopsy procedures will be performed by a registered dermatologist (Dr Paul Jarrett) and may cause slight discomfort
- I understand that the writing task may cause possible some sad emotions
- I understand that two samples of my skin will be stored at the University of Auckland and then sent to the University of Bristol for analysis and stored for a period of 6 years

Name:.............................................................................................................

Signature:.......................................................... Date:.........................

I wish to receive a copy of the results of this research       Yes / No

Email / postal address..................................................................................

APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON 16/08/2014 for 3 years, Reference Number 012656
Appendix C: Study 3 Ethics Approval, Participant Information Sheet and Consent Form

Office of the Vice-Chancellor
Finance, Ethics and Compliance

UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE (UAHPEC)

66-Oct-2015

MEMORANDUM TO:

Dr Elizabeth Broadbent
Psychological Medicine

Re: Application for Ethics Approval (Our Ref. 015904): Approved with comment

The Committee considered your application for ethics approval for your project entitled Skin barrier recovery and social support.

Ethics approval was given for a period of three years with the following comment(s):

Please amend the following:

1. PIS and CF
   a. Please consider informing the participants that the saliva samples will be labelled with the participants’ ID code only.

2. CF
   a. Please amend the sentence about withdrawal of data to the following: “I can withdraw any data traceable to after completing the study, in which case the data will be securely destroyed.”
   b. Please complete the incomplete sentence at the end of the document: “Please provide an email address or mailing address if you wish to receive a summary of the”
   c. Please add the UAHPEC approval wording: Approved by the University of Auckland Human Participants Ethics Committee on ...... for three years, Reference Number ..........

The expiry date for this approval is 06-Oct-2018.

If the project changes significantly you are required to resubmit a new application to UAHPEC for further consideration.

In order that an up-to-date record can be maintained, you are requested to notify UAHPEC once your project is completed.

The Chair and the members of UAHPEC would be happy to discuss general matters relating to ethics approvals if you wish to do so. Contact should be made through the UAHPEC Ethics Administrators at re-ethics@auckland.ac.nz in the first instance.

All communication with the UAHPEC regarding this application should include this reference number: 015904.
Participant Information Sheet

Dear Participant,

This form will provide you with information as to whether you wish to take part in this study exploring the effect tape stripping stress on skin healing after a tape stripping procedure.

This project is being carried out by Hayley Robinson, a PhD candidate (Department of Psychological Medicine, University of Auckland), Abhimati Ravikulan, summer student (Department of Medicine, University of Auckland), Dr Paul Jarrett (Consultant dermatologist, Middlemore Hospital) and Dr Elizabeth Broadbent (Department of Psychological Medicine, University of Auckland), who will be supervising the project.

It is important that you read this document carefully so that you can make an informed decision about whether you would like to participate.

Purpose of the study: Previous research has found that a social support can influence health outcomes and pain, but little research has been done to assess healing how social support may influence healing. The aim of the study is to investigate the effects of social support on skin recovery after tape stripping in the laboratory.

Eligibility: We are recruiting non-smoking participants who are aged 18 years or over and speak English. Approximately one hour and a half to two hours is required to complete the study at the Clinical Research Centre.

What would happen if you chose to participate? If you chose to participate in this research you will be asked to come to the University of Auckland to complete a session in the laboratory. Upon signing up to the study you will be randomised to either a social support condition or a control condition.

Questionnaires During this session, first you will be asked some information about your demographics, health behaviours and complete measures about your stress levels and mood. You will be asked to complete the same measures on stress at 3 other time points throughout the session.
Physiological measures
We will collect a salivary sample at the beginning (after a rest period of 30 minutes) and at three other time point in the study (before the tape stripping procedure, after tape stripping and at the end of the study session). These will be analysed for measures of cortisol and alpha amylase, an indicator of autonomic nervous system functioning. The samples will be stored in salicap containers (a small tube) in a secure lab in the University of Auckland at -20 degrees Celsius for 3 months, using usual protocols. The samples will be sent overseas to the University of Marburg (Germany) for analysis by a specialist. After the salivary samples have been analysed they will be disposed of. They will be labelled with an ID code—not your name.

Social support task
If randomised to the social support condition you will take part in a task that will help you to get to know another person participating in this experiment for 15 minutes. If you are randomised to the control condition you will quietly read for 15 minutes

Tape stripping
You will then undergo a tape stripping procedure whereby tape is applied to your skin on your forearm in three 1cm diameter areas just below your elbow. This removes the top layer of your skin. This will be repeated a number of times until your skin barrier function reaches a certain point. To ensure the right level is reached a small probe (1cm in diameter) will be pressed gently against your skin for a 2 minutes or less. After a 30 minute recovery period, at the end of the study skin barrier function will be measured again using the probe to see how much your skin has recovered.

Your rights as a participant: Participation in the study is entirely voluntary. If you choose to participate, you can change your mind at any time without giving a reason and without any negative consequences. You can withdraw from the study at any time and withdraw any data traceable to you until you have finished the study. Whether or not you participate in this study will not affect your relationship with the researchers. You will be given a copy of this document to keep.

Koha: You will receive a $20 Westfield voucher at the end of the session as koha for agreeing to participate in this research. You will receive this irrespective of whether you withdraw during the study.

Risks and discomforts: The procedures outlined in this protocol are minimally invasive and have been performed in other research settings. The tape stripping procedure may cause slight discomfort, and redness of the skin but this should disappear within 24 hours. If you have any allergy to cellotape or adhesives you should not participate in this research. If you have any current inflammatory skin diseases, chronic illness, immunological-related health problems or are taking medication that affect immune functioning you should not take part in this research. If skin irritation persists you should contact University Health Services on 09 923 7681 to make an appointment with a doctor, or Hayley Robinson at 09 3737599 Ext. 89454 to organise to see the study dermatologist, Dr Paul Jarrett.

Data storage: All data (questionnaire, salivary samples and skin barrier function data) will be stored in electronic format by the researcher. Paper questionnaires and consent forms will be stored in a locked filing cabinet in the researcher’s office at the University, and will be kept for a period of six years. Salivary samples will be stored in a freezer at the University of Auckland.

Confidentiality: All personal information will remain strictly confidential and no material that could personally identify you will be used in any report on this study. Participant names will only appear on the consent form, which will be coded with a participant identification number so that your identity
is kept confidential on all questionnaires and physiological data files. After completion of the study, all confidential data, including computer data files, will be kept for a minimum period of six years to allow for publication and re-analysis, after which time it will be securely and confidentially disposed of. Research publications and presentations from the study will not contain any information that could personally identify you.

**Results:** A summary of the results of this study will be sent to you if you wish. As it takes some time to analyse the results of studies, it may be more than a year after your participation that you receive this information.

**We appreciate the time you have taken to read this information. If you have any questions please contact:**

**Abhimati Ravikulan**  
Summer student, Department of Psychological Medicine,  
The University of Auckland  /Private Bag 92019, Auckland 1142  
Email: arav175@aucklanduni.ac.nz

**Hayley Robinson**  
PhD student, Department of Psychological Medicine,  
The University of Auckland  /Private Bag 92019, Auckland 1142  
Email: hrob458@aucklanduni.ac.nz  
Phone: +64 9 3737599 Ext. 89454

**Alternative contacts:**  
Dr Liz Broadbent, Department of Psychological Medicine,  
The University of Auckland  /Private Bag 92019, Auckland 1142  
Email: e.broadbent@auckland.ac.nz  
Phone: (09) 3737599 Ext. 86756

**Head of Department:**  
Associate Professor Sally Merry, Department of Psychological Medicine, The University of Auckland  
Email: s.merry@auckland.ac.nz

**For any concerns regarding ethical issues you may contact:**  
The Chair,  
The University of Auckland Human Participants Ethics Committee,  
The University of Auckland,  
Research Office,  /Private Bag 92019,  
Auckland 1142.  
Telephone 09 373-7599 ext. 83711.  
Email: ro-ethics@auckland.ac.nz

**APPROVED BY THE UNIVERSITY OF AUCKLAND HUMAN PARTICIPANTS ETHICS COMMITTEE ON**  
06/10/2015 for 3 years, Reference Number 015904
Project title: Skin barrier recovery and social support

Names of researchers: Dr Elizabeth Broadbent (supervisor), Dr Paul Jarrett (co-supervisor), Hayley Robinson (PhD candidate), Abhimati Ravikulan, (summer student)

Written Consent Form

I have read and understood the Participant Information Sheet, have understood the nature of the research, and know why I have been selected. I have had an opportunity to ask questions and have had them answered to my satisfaction.

Consent forms will be held for six years in accordance to university policy

- I agree to take part in this research.
- I understand that taking part in this research is voluntary (my choice).
- I understand that participation will take approximately an hour and a half.
- I understand that I am able to withdraw from the study at any time without giving a reason. I can withdraw any data traceable me after completing the study, in which case the data will be securely destroyed.
- I understand that the overall results may be published in a scientific journal but will not include any information that could identify me.
- I am aware that as a result of taking part in this study I will be given a $20 Westfield voucher as koha for agreeing to take part in this research irrespective of whether I complete the study.
- I understand that the research data will be stored for 6 years after which it will be destroyed.
- I understand that salivary samples will be stored securely at the University of Auckland at -20 degrees Celsius and will be sent to the University of Marburg (Germany) for analysis after which they will be disposed. They will be labelled with an ID code-not my name.
- I understand that the tape stripping procedure may cause slight discomfort, and redness of the skin but this will disappear within 24 hours. If skin irritation persists I understand I can contact University Health Services on 09 923 7681 to make an appointment with a doctor, or Hayley Robinson at 09 3737599 Ext. 89454 to organise to see the study dermatologist, Dr Paul Jarrett.
- I am not aware of any reason why I should not participate in this research

Name:..........................................................................................................................

Signature:.................................................. Date:.........................

I wish to receive a copy of the results of this research  Yes / No

Please provide an email address or mailing address if you wish to receive a summary of the results: ________________________________________________
Appendix D: Measures included in Study 1, 2 and 3

Background Information

Please answer the following questions by filling in the blanks or circling the answer that best correspond to you:

<table>
<thead>
<tr>
<th>1. Are you male or female?</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. How old are you?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Height (cm)</td>
<td>Weight (kg)</td>
<td></td>
</tr>
</tbody>
</table>

4. What ethnic group do you belong to? *Circle the numbers that apply to you*

<table>
<thead>
<tr>
<th>New Zealand European</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Māori</td>
<td>2</td>
</tr>
<tr>
<td>Samoan</td>
<td>3</td>
</tr>
<tr>
<td>Cook Island Māori</td>
<td>4</td>
</tr>
<tr>
<td>Tongan</td>
<td>5</td>
</tr>
<tr>
<td>Niuean</td>
<td>6</td>
</tr>
<tr>
<td>Chinese</td>
<td>7</td>
</tr>
<tr>
<td>Indian</td>
<td>8</td>
</tr>
<tr>
<td>Other (such as Dutch, Japanese, Tokelauan)</td>
<td>9</td>
</tr>
<tr>
<td>Please specify:</td>
<td></td>
</tr>
</tbody>
</table>

5. At what level did you complete your formal education? (circle number)

<table>
<thead>
<tr>
<th>Primary school</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary school (up to Year 10)</td>
<td>2</td>
</tr>
<tr>
<td>Secondary school (including Year 11)</td>
<td>3</td>
</tr>
<tr>
<td>Secondary school (including Years 12 &amp; 13)</td>
<td>4</td>
</tr>
<tr>
<td>Technical or Trade Certificate</td>
<td>5</td>
</tr>
<tr>
<td>University or Polytechnic Diploma</td>
<td>6</td>
</tr>
<tr>
<td>University degree</td>
<td>7</td>
</tr>
</tbody>
</table>
Heath-related behaviours

1. Do you currently smoke?
   □ Yes on an average day I smoke______ cigarettes
   □ No, not any more. I quit smoking __________ ago.
   □ No, I have never smoked

2. During the past three months how often have you drunk alcohol, on average?
   □ Not at all
   □ 1-2 times a week
   □ Less than once a month
   □ 3-6 times a week
   □ 1-3 times a month
   □ Everyday

3. On days when you did drink alcohol in the last three months, how many drinks did you have on an average day?
   □ 0 drinks
   □ 1-2 drinks
   □ 3-4 drinks
   □ 1 drink
   □ 5-6 drinks
   □ 2 drinks
   □ 7-11 drinks
   □ 12 or more drinks

4. During your average week, how many times do you engage in 30 minutes or more of physical activity (e.g. going for a walk, going to the gym, riding a bike, swimming)?
   □ Never
   □ Three times
   □ Six times
   □ Once
   □ Four times
   □ Everyday
   □ Twice
   □ Five times

5. During the past week, how would you rate your diet?
   1 Very poor
   2 Poor
   3 Fair
   4 Good
   5 Very good
Sleep (taken from Pittsburg Sleep Quality Index (Buysse, Reynolds, Monk, Berman & Kupfer, 1989))

1. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed)

   HOURS OF SLEEP PER NIGHT __________

2. During the past month, how would you rate your quality of sleep?

   1. Very poor
   2. Poor
   3. Fair
   4. Good
   5. Very good

3. During the past 24 hours how many hours sleep have you had? __________
Perceived Stress Scale (PSS; Cohen & Williamson, 1988)

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by circling how often you felt or thought a certain way.

0 = Never   1 = Almost Never   2 = Sometimes   3 = Fairly Often   4 = Very Often

In the last month, how often have you been upset because of something that happened unexpectedly? ................................................. 0 1 2 3 4

In the last month, how often have you felt that you were unable to control the important things in your life? ................................. 0 1 2 3 4

In the last month, how often have you felt nervous and “stressed”? .......................................................................................... 0 1 2 3 4

In the last month, how often have you felt confident about your ability to handle your personal problems? ................................. 0 1 2 3 4

In the last month, how often have you felt that things were going your way? ................................................................. 0 1 2 3 4

In the last month, how often have you found that you could not cope with all the things that you had to do? ............................... 0 1 2 3 4

In the last month, how often have you been able to control irritations in your life? ................................................................. 0 1 2 3 4

In the last month, how often have you felt that you were on top of things? ................................................................................ 0 1 2 3 4

In the last month, how often have you been angered because of things that were outside of your control? ................................. 0 1 2 3 4

In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?................. 0 1 2 3 4
Affect Valuation Index (AVI; Tsai, Knutson & Fung, 2006)

Listed below are a number of words that describe feelings. Some of the feelings are very similar to each other, whereas others are very different from each other. Read each word and then rate how much you feel that emotion right now using the following scale:

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
<td>A little</td>
<td>Moderately</td>
<td>Quite a bit</td>
<td>Extremely</td>
</tr>
</tbody>
</table>

In this moment I feel...

enthusiastic _____ astonished _____ nervous _____
dull _____ quiet _____ relaxed _____
excited _____ surprised _____ elated _____
sleepy _____ still _____ lonely _____
strong _____ passive _____ content _____
sluggish _____ inactive _____ sad _____
euphoric _____ fearful _____ happy _____
idle _____ calm _____ unhappy _____
aroused _____ hostile _____ satisfied _____
rested _____ peaceful _____ serene _____
Visual Analogue Scales (VAS)

Included in Study 1

Please rate how you feel at the moment on the three scales below by putting an X on the appropriate place on the line.

Not at all relaxed | Extremely relaxed

No sensation of pain | Most sensation of pain imaginable

Included in Study 3

Please rate how you feel at the moment on the three scales below by putting an X on the appropriate place on the line.

Not at all stressed | Extremely stressed
Appendix E: Expressive and control writing instructions

(Pennebaker, Colder & Sharp, 1990)

Instructions A

Today and for the next two days, we would like you to write about your day-to-day activities. For each session, you will have a different writing topic relating to the way you spend your time.

In your writing, the most important thing is to be as objective and descriptive as possible (i.e. just the facts, no emotions, no opinions, no feelings). Concentrate on the specific details of your activities, even when referring to everyday tasks. You may include things such as the time you get up in the morning and go to bed at night, meals you have eaten or prepared, any meetings, appointments, or social activities you attended, including details about what you did (i.e. had coffee), who you saw (i.e. cousin Ann), where you met (i.e. Café Paris), any television programmes you watched, and any work-related or domestic tasks you have done.

**Today:** Please write about what you did last week.

**Tomorrow:** Please write about what you did over the past 24 hours.

**On the last day:** Please write about your plans for the upcoming week.

Try to write uninterrupted (non-stop) for the full 20 minutes (e.g. find a quiet place, shut the door, turn off your phone, radio, and television). If you run out of things to say, just repeat what you’ve already written. Don’t worry about spelling or grammar.

Once you are finished writing each day, please fill out the ‘post-writing questionnaire’.
Instructions B

Today, and for the next two days, we would like you to write about your deepest thoughts and feelings about the most traumatic, upsetting experience of your entire life. If you do not have a traumatic experience, please write about significant life-changing events that you have recently experienced, or are currently experiencing. Ideally, we would like you to write about significant experiences or conflicts that you have not previously discussed in great detail with others. You might tie your personal experiences to other parts of your life, such as how they are related to your childhood, your parents, people you love, who you are, or who you want to be. You might choose to write about your health and upcoming surgery. Whatever you decide to write about, it is very important that you really delve (dig deep) into your deepest emotions and thoughts. We really want you to let go and explore your very deepest feelings and thoughts.

Please try to write uninterrupted (non-stop) for the full 20 minutes (e.g. find a quiet place, shut the door, turn off your telephone, radio, and television). If you run out of things to say, just repeat what you’ve already written. Don’t worry about spelling or grammar.

Remember, you have three (3) days to write over the next week. You can write about the same experience on all three days, or you can write about different experiences each day. Once you are finished writing each day, please fill out the ‘post-writing questionnaire’
Post Writing Questionnaire

Once you have finished writing your essay for today, please answer the following question. Please **circle** the answer that best describes how you feel:

Day 1

Date: ________________  Minutes spent writing: _______

Overall, how much did you reveal your emotions in what you wrote today?

Not at all  A little  Somewhat  Quite a bit  A great deal

Day 2

Date: ________________  Minutes spent writing: _______

Overall, how much did you reveal your emotions in what you wrote today?

Not at all  A little  Somewhat  Quite a bit  A great deal

Day 3

Date: ________________  Minutes spent writing: _______

Overall, how much did you reveal your emotions in what you wrote today?

Not at all  A little  Somewhat  Quite a bit  A great deal
Appendix F: Relationship Closeness Induction Task (RCIT; Sedikides, Campbell, Reeder & Elliot, 1999)

Conversation Task

Instructions (read to participants)
You and the other participant will receive 3 identical lists of questions. The lists will be on separate pages. We would like you to engage as natural a conversation as possible using these questions. Please take turns asking and answering the questions.

One participant should ask the first question on the list. The other participant should answer and then ask the same question to the first person.

There is a time limit on each of the 3 lists of question. You should try and finish all the questions in that time limit

Check off each question you finish.

You may spend 2 minute on the first list of questions, 5 minutes on the second list and 8 minutes on the third list.

I will come in and tell you when time is up and you can go onto the next set of questions. When this occurs, finish the question you are on and go onto the next list.

This conversation is confidential. Please do not discuss it outside of this setting in order to protect one another’s privacy

List 1

Please tick once each question is completed

1. What is your first name?  
2. How old are you?  
3. Where are you from?  
4. What year are you at University OR What do you do if you are not at University?  
5. What are your hobbies?  
6. What is your dream job?  

List 2

Please tick once each question is completed

1. What would be the perfect lifestyle for you?  
2. What is something you have always wanted to do but probably will be never able to do?  
3. If you could travel anywhere in the world, where would you go and why?  
4. What is one strange thing that has happened to you over the past year?  
5. What is one embarrassing thing that has happened to you over the past year?
6. What is one thing happening in your life that makes you stressed out?
7. If you could change one thing that happened to you in high school, what would that be?
8. If you could change one thing about yourself, what would that be?
9. What is one habit you would like to break?
10. If you could have one wish granted, what would it be?
11. Is it difficult or easy for you to meet people? Why?
12. Describe the last time you felt lonely.

List 3

Please tick once each question is completed

1. What is one emotional experience that you have had with a good friend?
2. What is one of your biggest fears?
3. What is your most frightening early memory?
4. What is your happiest early childhood memory?
5. What is one thing about yourself that most people would consider surprising?
6. What is one recent accomplishment that you are proud of?
7. Tell me one thing about yourself that most people who already know you don’t know.
8. Given the choice of anyone in the world, whom would you want as a dinner guest?
9. If a crystal ball could tell you the truth about yourself, your life, the future or anything else, what would you want to know?
10. Your house, containing everything you own, catches fire. After saving your loved ones and pets, you have time to safely make a final dash to save any one item. What would it be? Why?


After conversation task

Please answer 'Yes' or 'No' to these 6 questions based on the conversation you just had.

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you think you had adequate privacy in your conversation?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did you feel relatively comfortable in this conversation setting?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you consider conversation a good way to get to know somebody?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you often engage in conversations similar to the one you just engaged in?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you think the majority of your friends would ask questions similar to those asked in this conversation?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you think your friends consider conversation the most important way to get to know somebody?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For the next questions please indicate your answers on the scales

1. How close do you feel to the participant with whom you are working on this study?

   1-not at all close   3   6   9-very close

2. How similar do you feel to the participant with whom you are working on this study?

   1-not similar at all   3   6   9-very similar
3. How much do you like the participant with whom you are working on this study?

1- not at all  |  3  |  6  |  9-very much

4. In the future, to what extent do you feel you could be friends with the participant with whom you are working on this study?

1- not at all  |  3  |  6  |  9-very much