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Engineered systems to study the synergistic signaling between integrin-mediated mechanotransduction and growth factors (Review)

Isabela Monteiro A.,[1,2] Tarek Kollmetz,[1,2] and Jenny Malmström[1,2,a)]
[1]*Department of Chemical and Materials Engineering, The University of Auckland, Auckland 1023, New Zealand*
[2]*MacDiarmid Institute for Advanced Materials and Nanotechnology, Wellington 6140, New Zealand*

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While tremendous leaps in knowledge into cellular signaling and control have been achieved over the last few decades, there is still more to learn in how different signaling pathways act synergistically. A better understanding and control of cells *in vitro* and *in vivo* is important to enable more successful and safe applications of tissue engineering and stem cell therapy. This review is focused on two central ways cells sense their surroundings, namely, integrin-mediated mechanotransduction and growth factor signaling. Specifically, the authors explore how engineered interfaces have been applied to learn more about these processes, and how these important signaling pathways interact synergistically.
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I. INTRODUCTION

Humans have a remarkable ability to heal and renew tissues. To achieve this, a multitude of stimuli act on cells *in vivo*, to steer their survival, proliferation, migration, and differentiation. A deeper understanding of how cells respond to different stimuli is still needed to answer fundamental biological questions and to differentiate between signals leading to healthy versus disease states. The tight regulation of stem cells *in vivo* is a particularly interesting area of study as the use of stem cells presents unprecedented potential in regenerative medicine. However, the success of such therapies is hampered by the gap in knowledge of cell fate and a lack of understanding of stem cell behavior *in vitro*. A better understanding of stem cell behavior will lead to an increased and safer use of stem cells in tissue engineering and the treatment of degenerative diseases. Although individual signaling pathways in cells are often well understood, synergistic effects leading, for example, down a particular differentiation pathway need further research to be fully mapped and understood.

Receptors in the cell membrane are a vital part of how cells sense and respond to their environment. The binding between a ligand and its receptor is highly specific, but many factors influence both the binding and the downstream effects of the binding. The receptor may need to be activated to be available for binding, by, for example, clustering or phosphorylation. The ligand may also need to first be released from the extracellular matrix (ECM) that surrounds the cells in our tissues by degrading or pulling on the matrix.[1–3] The downstream signaling effects from several receptor types can furthermore interact in a synergistic or inhibitory fashion.[4,5] Cells sense and adapt to forces and physical constraints imposed by the ECM. This so-called mechanotransduction is defined as the processes through which cells convert mechanical stimuli that they sense, to biochemical signals that lead to specific cellular responses. It plays a crucial role in cell function, differentiation, and cancer.[6–10] In this review, we specifically consider integrin-mediated mechanotransduction, excluding other mechanosensitive complexes such as adherens junctions and the linker of nucleoskeleton and cytoskeleton complex. The ECM mechanical properties and distribution of ligands are both sensed and modulated through the contractile and adhesive molecular machinery in the cells. This machinery consists of bonds between the ECM and transmembrane integrin receptors, adaptor proteins, and the force generating actomyosin cytoskeleton, often referred to as a molecular clutch.[10–12] Growth factors (GFs) also bind to receptors in the cell membrane and are vital for a range of cellular responses, such as proliferation and differentiation.

When cells adhere to the customary 2D hard surfaces in *in vitro* cell culture, they generally become well spread with defined adhesion points connected to the actomyosin network. By carefully controlling this cell-material interface *in vitro*, an understanding can be built up of how separate surface cues guide cellular behavior.[13–15] Since it is well established that cell responses are complex and interconnected, research is currently directed toward understanding the synergistic effects between mechanotransduction and GF signaling.[11,16,17] Much of the work to understand intricacies in cell biology is performed in engineered *in vitro* model systems, where individual factors can be carefully studied. Challenges still remain to design model systems able to study synergistic effects in a systematic manner, while still producing results of relevance to the *in vivo* environment. Figure 1 illustrates the important biological length scales from cellular, via subcellular, to the scale of individual molecules—relevant to the design of engineered model systems.

There are many recent reviews on topics such as mechanotransduction, cell adhesion, extracellular matrix signals,

a)Electronic mail: j.malmstrom@auckland.ac.nz

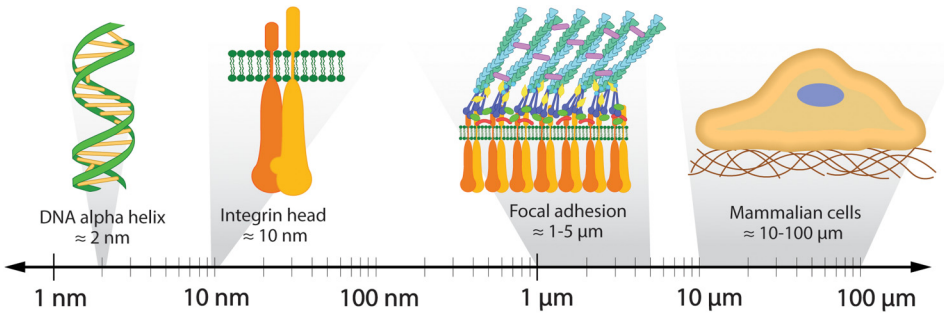

FIG. 1. Simplified illustration of the length scale of cellular components, starting from the nanometer scale of our genetic material and single integrin heads, to the micrometer range of mature focal adhesions and entire mammalian cells. This length scale fits well with engineered structures that can be produced by micro- and nanofabrication.

growth factors, and engineered surfaces presenting both cell adhesion ligands and growth factors.[16,18–24] In this review, our aim is to provide a condensed account of the important biological molecules and pathways that the surface engineer needs to have knowledge of to design new interfaces to influence cell biology. The review then discusses various approaches of such engineered model systems used to date, and examples of how they have been used to unravel intricate details about mechanotransduction and growth factor signaling. Taken together, we seek to provide an understanding of mechanotransduction and growth factor signaling and how these separate signaling systems can act synergistically to understand and steer cellular behavior.

This review begins by providing an introduction to the important molecules and signaling pathways involved in both mechanotransduction (Sec. II) and growth factor signaling (Sec. III). Once this basis is established, we review how engineered materials and surfaces can be applied to further understanding of mechanotransduction (Sec. IV) and growth factor signaling (Sec. V). Finally, in Sec. VI, we look at how these two biological signals are known to interact and how new engineered materials have been applied to investigate such synergies. Finally, the review finishes with a conclusion and outlook discussing some of the present challenges in the field.

II. MECHANOTRANSDUCTION

The natural cellular environment contains a range of biophysical signals that cells sense and respond to. The sensing of the environment takes place through dynamic binding and unbinding between the cell and its environment. Integrins are transmembrane receptors for the extracellular matrix that allow for the transmission of physical signals, from the outside of the cell to the inside, in a process called mechanotransduction.[25] This process directs cell mobility,[26] differentiation,[11] tissue remodeling, homeostasis, and even cancer progression.[27] To fully understand the significance and influence of mechanical signals in cell behavior, it is important to first comprehend how cells exert forces on substrates, and how the forces are transmitted through the cytoskeleton and trigger signaling cascades that affect cell function. Sections II A and II B aim to introduce the main macromolecules and signaling pathways in the process of mechanotransduction.

A. Key molecules involved in mechanotransduction

The structures responsible for mechanical linkage and regulatory signaling between cells and the ECM are focal adhesions (FAs). In the early stages of cell spreading and migration, force-independent and transient adhesion structures called focal complexes or nascent adhesions are formed. The earliest integrin clusters (of about 50 integrins) form irrespectively of matrix stiffness.[28] On rigid substrates, these nascent adhesions are mainly found at the periphery of cells and are transient structures either disassembling or maturing into FAs, which are force-dependent and relatively stable compared to nascent adhesions.[29,30] FAs are large, dynamic macromolecular assemblies that form a <150 nm high plaque[31] containing over 100 different proteins, which suggests an abundant functional diversity.[32] FAs do much more than just anchoring cells to the underlying ECM, they also transmit force through the adhesions, initiating multiple intracellular signaling pathways able to regulate gene expression, and consequently cell growth and survival.[32,33] The most central player in the focal adhesions are the integrins (heterodimeric transmembrane proteins, consisting of noncovalently associated α and β subunits). The combination of integrin α and β subunits specifies the type of ECM proteins they bind to, and with what binding affinity and, consequently, different cells produce different types of integrins.[29,34] Therefore, the integrin subunit combination critically determines the cellular response to the surrounding.[35]

The integrins themselves have no signaling domain, but when an integrin receptor binds to its ECM ligand and is activated, intracellular proteins are recruited and assemble around the activated cytoplasmic domain, leading to an integrated process between chemical and mechanical signals. A considerable number of adaptor molecules participate in the process as structural or regulatory components,[36] forming the molecular clutch, a molecular bridge that vertically connects integrin receptors with actin filaments in the cell cytoplasm.[25] Immunofluorescence and super-resolution light microscopy studies have been instrumental in understanding this molecular complex. It has been found that the focal adhesion core

region is localized in an ~40 nm gap between integrins and actin, described as a laminated structure of three mechanically linked functional layers: An integrin signaling layer juxtaposed to the cell membrane, a middle force transduction layer, and an actin regulatory layer[37] (Fig. 2).

While FAs are dynamic structures that change their size and composition with maturation (driven by time, ligands, and force generation),[38] one simple model of focal adhesion architecture is as follows: The integrin signaling layer extends for ~20 nm from the plasma membrane, containing the cytoplasmic integrin tail, the talin head domain (THD), Focal Adhesion Kinase (FAK), and paxillin. FAK and paxillin depend on integrin-ECM gathering for activation and engagement in signaling cascades that control adhesion dynamics.[37] Talin and vinculin are located in the force transduction layer, where diagonally arranged talin proteins make a direct connection between integrins and f-actin. Vinculins bind along the talin rod domain and f-actin, anchoring the actin filaments to the cell membrane, and thus reinforcing tension within the focal adhesions. Both talin and vinculin have been indicated as central to the force transmission linkages in the FA, thus defining the force transduction layer.[39,40] Lastly, the actin regulatory layer connects to the actin stress fibers via vasodilator-stimulated phosphoprotein (VASP) and zyxin, with roles in actin dynamics and bundling regulation.[41,42] α-Actinin localizes predominantly along the actin stress fibers where it may stabilize FAs as tensile force increases,[37] but has also been found capable to directly link integrin to actin, in competition with talin, in particular in mature FAs.[43] An interesting recent observation by Spiess *et al.*[44] suggests another level of organization within FAs, with active and inactive integrins seen to separate into nanoclusters within the adhesion. Movement and dynamics of integrins within FAs have also been found to be subtype dependent with a more stationary role played by β_3 integrins compared to β_1.[45] It is worth mentioning that the highly dynamic nature of FAs means that many different compositions are possible. Variations to the above described model are dependent not only on time and force generation, but also on the integrin subtypes, the ligands, and the cellular environment (in particular, 2D versus 3D culture).[38,43,46,47]

The majority of the proteins mentioned above are considered cytoskeletal proteins due to their direct or indirect function in linking integrins to actin and due to their lack of enzymatic activity. However, there are also many biochemically active proteins associated to FAs, such as nonreceptor kinases, or adaptor proteins that can recruit these molecules.[48] This, and the fact that many of the FA proteins are regulated by phosphorylation, leads to an intricately fine-tuned biological machinery. It is important to note that not all the associated components are found in all cell-matrix adhesion complexes. Many of them may be expressed in a specific cell type and others may be associated differently depending on the FA maturity.[49] In fact, proteomic analysis of isolated integrin adhesions has revealed a staggering complexity, identifying over 2000 potentially involved proteins. Horton *et al.*[50] used bioinformatics to identify core components of a so-called "consensus adhesome," providing a useful recourse for the scientific community.[50]

The innermost region of FAs is connected to double right-handed helical filaments of actin. These filaments are composed by aggregation and polymerization of the actin monomers, G-actin, into its linear filamentous state, F-actin, and form a larger-scale network known as the actin cytoskeleton.[29] This dynamic structure plays a central role in cell shape and motility and serves as transportation trail inside the cells, extending throughout the cytoplasm from the nucleus to the plasma membrane.

B. Signaling pathways in mechanotransduction

As mentioned before, integrin-ECM binding leads to downstream signals that up- or down-regulate intracellular processes such as transcription factor activation and gene expression.[40,51] One interesting force-dependent mechanism for protein activation is the ECM/integrin/talin/F-actin signaling

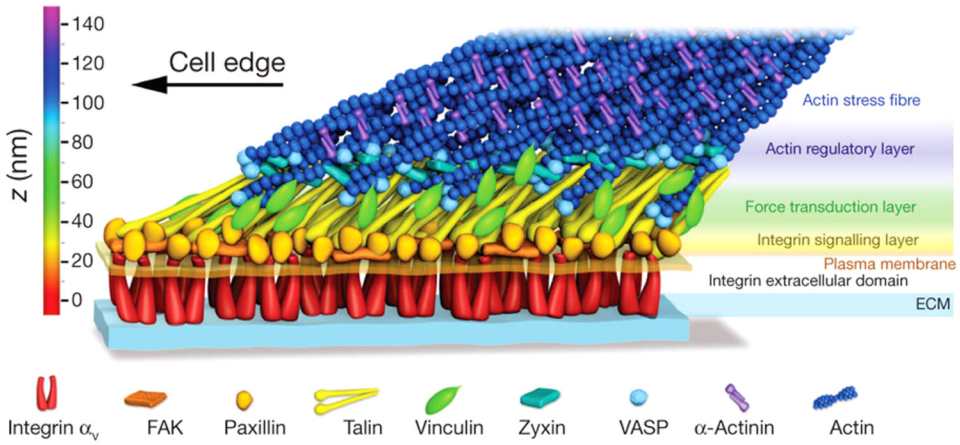

FIG. 2. Schematic model of focal adhesion molecular architecture, showing the three horizontal layers of the FA core: the integrin signaling layer, the force transduction layer, and the actin regulatory layer. Reprinted with permission from Kanchanawong *et al.*, Nature **468**, 580 (2010). Copyright 2010, Springer Nature.

axis, with activation relying mostly on conformational changes caused by stretching during FA formation, allowing protein–protein interactions and enzymatic reactions.[52,53] The talin N-terminal domain, also called the THD, binds to the cytoplasmic integrin tail, and the long C-terminal rod domain binds directly to the F-actin network. It has been demonstrated that force-dependent unfolding of the talin rod domain by actomyosin contraction exposes many binding sites for vinculin, actin, and other regulatory proteins.[53–55] When cells are cultured on soft substrates, the forces on this molecular clutch are low and insufficient to unfold the talin rod domain before rupture of the integrin-ECM bonds, thus no further recruitment of focal adhesion proteins occur. On stiff substrates, cell-ECM force transmission is higher, inducing talin stretching and further clustering of structural molecules that are essential to trigger downstream signaling pathways and further focal adhesion maturation.[12]

Integrins have no enzymatic activity themselves, but their β subunits connect to the signal-transducing kinase, FAK, directly or indirectly through vinculin or paxillin.[56] FAK is a central component of the signaling initiated through mechanosensing, as it mediates activity of many others proteins. FAK is involved in downstream signaling cascades that include Src (nonreceptor tyrosine kinase family), Rho [small GTPase, activating Rho-associated protein kinase (ROCK)], and ERK (extracellular signal-regulated kinases), which have important roles in matrix stiffness responses.[57] Therefore, FAK is a potential signaling target to inhibit cellular responses to matrix mechanical cues, and its dysregulation is associated with cancer invasion and metastasis.[58] An increase in matrix stiffness induces increase of FAK phosphorylation at Tyr397, which is necessary for Src binding, and complete FAK activation.[58,59]

When the cytoskeleton is assembled, and already under some tension, it provides very fast mechanical signal transmission. In fact, it has been found that mechanical stress can very rapidly propagate from integrin adhesion sites, along the actin stress fibers, to induce the kinase activity of Src far away from the site of the applied mechanical stress.[60] The speed of such propagation is much higher than what can be achieved by diffusion in the crowded cytoplasm or by motor protein-mediated transport of signaling molecules.[61]

Another important pathway for transduction of mechanical signals is the Rho/ROCK pathway, critical regulator of cytoskeletal dynamics, cellular contractility, and motility through the formation of actin stress fibers. Integrin engagement with the ECM activates the Rho guanine nucleotide exchange factors, GEF-H1 and LARG, through ERK and Src family downstream signaling cascade, respectively, to further catalyze the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP), which directly activates Rho signaling.[62] Within the Rho family of GTPases, RhoA is involved in actin organization and myosin contractility through activation of its major downstream effector, ROCK (Rho-associated coiled-coil containing protein kinase).[63] ROCK phosphorylates different substrates including LIM kinase, myosin light chain (MLC), and MLC phosphatase and mediates the formation of actin stress fibers and focal adhesions in various cell types.[64] ROCK phosphorylates and activates LIM kinase, which in turn phosphorylates cofilin, thereby inactivating its actin-depolymerization activity.[65] Additionally, ROCK phosphorylates MLC of myosin II and inactivates MLC phosphatase, responsible for dephosphorylation of MLC, promoting the actin binding of myosin II and, thus, the generation of cellular force from actomyosin filament contraction.[64,66] The increase in actin contractility and formation of stress fibers leads to integrin and adaptor molecules clustering, resulting in focal adhesion maturation.[32] These downstream pathways are essential for optimal cellular tension and rigidity sensing.

The cellular response to mechanical properties of the ECM also occurs at the transcriptional level. Both YAP (Yes-associated protein) and TAZ (transcriptional coactivator with PDZ-binding motif) are mechanosensitive transcriptional activators with critical roles in cell development,[67] organ size growth,[68] and cancer[69] and have historically been described to exert overlapping/redundant functions.[70] Nardone *et al.*[71] recently demonstrated that YAP nuclear shuttling is a necessary downstream event of Rho/ROCK signaling to stabilize the anchorage of the actin cytoskeleton to the cell membrane and control FA formation. Moreover, YAP nuclear accumulation was seen to be controlled by cell area regardless of FA assembly. Once YAP is located in the cell nucleus, it activates the transcription of genes encoding for a variety of cell functions including a number of FA docking and cytoskeletal proteins. TAZ, however, was not found to affect FA dynamics.[71] Elosegui-Artola *et al.*[33] further clarified that force-dependent changes in nuclear pores trigger YAP translocation to the cell nucleus by reducing their mechanical restriction. This indicates that the result found by Nardone *et al.*[71] is caused by the applied forces to the nucleus that might stretch and curve nuclear pores, leading to increased YAP import. Additionally, it has been demonstrated that YAP nuclear translocation is only triggered above a substrate rigidity threshold (5 kPa) in a talin/vinculin-dependent manner,[12] but the mechanism by which this happen remains an open question.

In other words, these mechanotransduction pathways are engaged by the cells' external stress and mechanical environment, to which cells respond by regulating the biochemical signaling pathways in a constant feedback loop. Although many other signaling pathways are involved in the machinery of mechanosensitivity, the above discussed pathways are the most studied ones and are schematically represented in Fig. 3. In addition to mechanical cues, the signaling pathways initiated by external stimuli are also affected by the perception and processing of biochemical molecules, such as GFs. Growth factor-mediated signaling pathways are coupled in different ways with mechanotransduction pathways in order to control cell adhesion, proliferation, motility, and survival.

To summarize Sec. II, the transduction of force through the cell membrane occurs via adhesion complexes, such as focal adhesions. Integrins span the membrane and physically

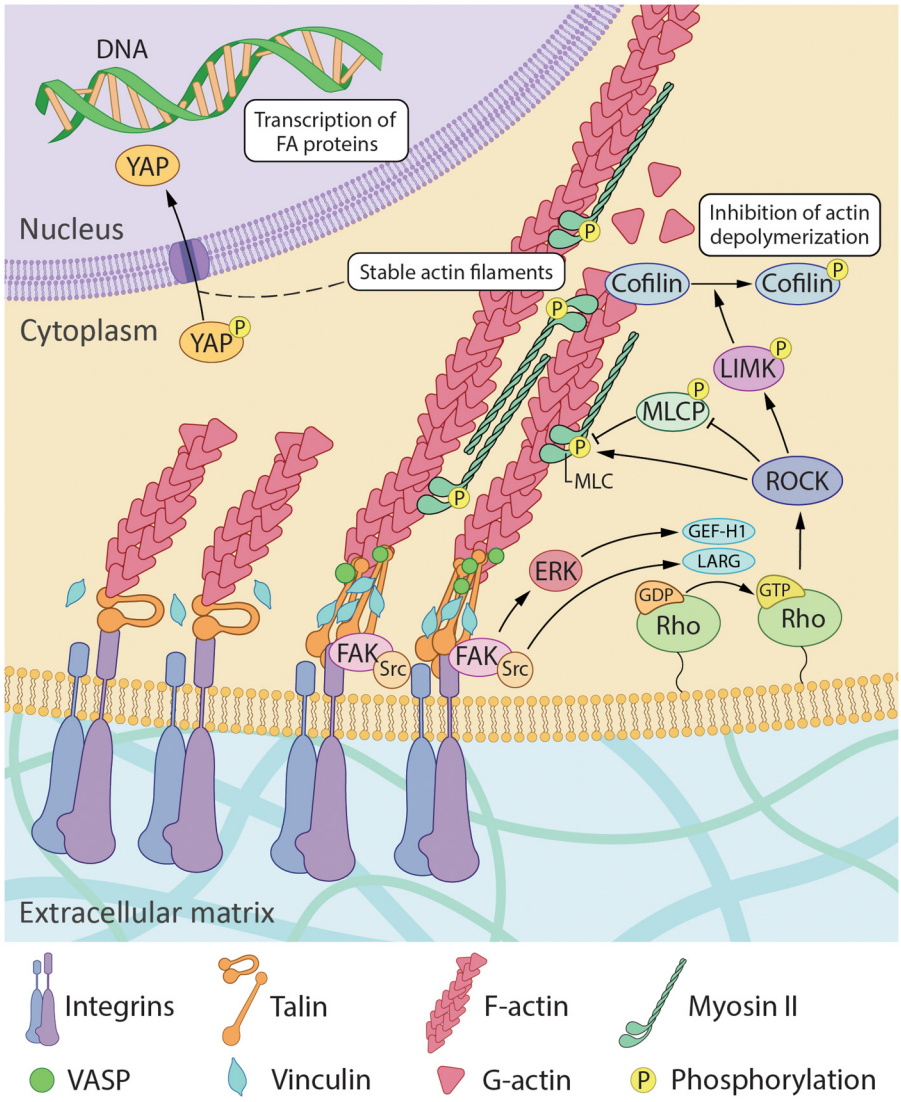

FIG. 3. Schematic outlining the major signaling pathways in mechanotransduction as discussed in Sec. II B.

transduce force to the actin cytoskeleton via a large number of intracellular proteins. These mechanical signals initiate biochemical signaling pathways via focal adhesion kinase and other kinases. These processes regulate, for example, cytoskeletal dynamics, cellular contractility, and motility. Mechanical signals can even directly influence gene transcription via mechanosensitive transcription factors.

III. GROWTH FACTORS

Growth factors were first discovered by the Italian developmental biologist Rita Levi-Montalcini and the American biochemist Stanley Cohen, which won them the 1986 Nobel Prize in Physiology or Medicine for their discovery of nerve growth factor (NGF) and epidermal growth factor (EGF), respectively.[72] These soluble, secreted, proteins are a way of communication between cells, and cells and their ECM, capable of stimulating cell signaling cascades that induce particular biological outcomes by binding to highly specific transmembrane receptors on the target cell. The mechanism by which the signal is transmitted from the cell membrane to the nucleus is complex, involving a combination of events such as protein phosphorylation, ion fluxes, changes in metabolism, transcription factor activation, gene expression, and protein synthesis, resulting in a coordinated biological response.[73] The impact of growth factors extends to control cell cycle, mobility, differentiation, tissue homeostasis as well as repair and maintenance (e.g., wound healing), regulating the growth and development of an organism.[11,74]

Growth factors have been classified into families based on their apparent activity and/or the type of their target cells, system, or tissue. Some of the most broadly studied families of growth factors are those relating to wound healing, angiogenesis, and osteogenesis. These include EGF, platelet-derived growth factor (PDGF), and transforming growth factor-beta (TGF-β), which are involved in the conversion of fibroblasts into myofibroblasts that generate a constrictive force along the ECM borders, facilitating wound closure.[74,75]

Vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) are angiogenic factors that are released in the ECM to stimulate endothelial cell proliferation and migration to form new blood vessels.[76] Bone morphogenetic proteins (BMPs), belonging to the TGF-β superfamily, are extensively used for inducing osteoblast differentiation and new bone formation in bone defects and play critical roles in heart, neural, and cartilage development.[77] Some well-known families of growth factors are summarized in Table I.

A. GF presentation by ECM

Growth factors are naturally secreted from cells and can exert their stimulation through autocrine or paracrine mechanisms. Due to their short half-lives and slow diffusion through the intercellular space, growth factors act relatively locally.[96] The slow diffusion is partly due to the high affinity of GFs to many ECM proteins.[97] Besides providing structural integrity to tissues and mediating cell attachment, the ECM also serves as a reservoir of GFs by sequestering and storing these molecules. This also helps to protect the GFs from degradation and presents them more efficiently to their receptors. The affinity of growth factors to ECM proteins can also have an inhibitory effect on growth factor signaling, by arresting them and blocking their interaction with their target receptors.[98] In other words, the ECM regulates the local concentration and consequently the biological activity of growth factors by allowing for spatiotemporal presentation of GFs to their receptors.[99,100]

Heparan sulfate proteoglycans (HSPGs) are linear polysaccharides with multiple heparan sulfate (HS) side chains covalently linked to the core protein[101] that can bind to a

TABLE I. Major growth factors families.

Name	Abbreviation	Examples	Source	Target cells	Action	Reference
Fibroblast growth factor family	FGF	FGF(1–23)	Pituitary, endothelial cells, fibroblasts, macrophages, mast cell, T lymphocytes	Endothelial cell, fibroblasts	Mitogenesis, mesoderm induction, neural development, tissue remodeling, angiogenesis, keratinocyte organization, and wound healing processes	78–81
Vascular endothelial growth factor family	VEGF	VEGF-(A-D), PlGF	Endothelial cells, keratinocytes, platelets, macrophages, fibroblasts	Endothelial cells	Mitogenesis, vasculogenesis, angiogenesis, lymphangiogenesis	80, 82, 83
Insulin-like growth factor family	IGF	IGF-1, IGF-2	Liver cells, fibroblasts, plasma	Osteocytes, neurons, myoblasts, myocytes, vascular smooth muscle, fibroblasts	Neurotrophic factors, skeletal muscle hypertrophy	84–86
Epidermal growth factor family	EGF	Epigen, TGF-α EPR, AREG, BTC, NRG(1–4)	Platelets, macrophages, keratinocytes, epithelial cells	Epithelial cells, fibroblasts, endothelial cells	Mitogenesis of epidermal and epithelial cell, osteogenic differentiation, angiogenesis	81, 87
Platelet-derived growth factor family	PDGF	PDGF-1, PDGF-2	Platelets, macrophages, fibroblasts, endothelial cells, keratinocytes, smooth muscle cells	Vascular smooth muscle, fibroblasts, monocytes, macrophages	Migration and mitogenesis of fibroblasts, smooth muscle cells, monocytes, mesenchymal and glial cells, angiogenesis	80, 81
Nerve growth factor family	NGF	NGF	Mast cells, neurons	Neurons	Neurotrophic factors, regulation of growth, proliferation, and survival of nerve cells, proliferation of pancreatic beta cells, regulation of the immune system	88–90
Hepatocyte growth factor family	HGF	HGF	Mesenchymal cells, platelets, fibroblasts, endothelial cells	Epithelial cells, endothelial cells, hematopoietic progenitor cells, T lymphocytes	Mitogenesis of epithelial cells, endothelial cells, hepatocytes, biliary epithelium cells and mammary glands, liver regeneration	81, 91
Transforming growth factor β family	TGF-β	TGF-β(1–3) GDFs, GDNF, BMP(2–7) Inh-β (A-C)	Macrophages, fibroblasts, keratinocytes, T lymphocytes, platelets, endothelial cells, smooth muscle cells, neurons	Fibroblasts, endothelial cells, keratinocytes, lymphocytes, monocytes, neurons, adipocytes	Inhibition of growth for most of the epithelial cells and leukocytes, proliferation of fibroblasts and smooth muscle cells, development of fibrosis, induce the formation of bone and cartilage, regulation of neuron survival, adipogenesis, regulation of the menstrual cycle, spermatogenesis	81, 92–95

variety of ligands, including growth factors, cytokines, chemokines, and morphogens, protecting them against proteolysis.[102] The major HSPGs presented in the extracellular matrix, particularly in basement membranes, include perlecan, agrin and collagen type XVIII while the main cell membrane bound HSPGs are the transmembrane syndecans and glypicans.[102] Some growth factor-binding sites have also been discovered within nonproteoglycan extracellular matrix proteins such as fibronectin,[103,104] fibrinogen,[105] tenascin C,[106] collagen IV,[107] and vitronectin.[108] For growth factors bound to the ECM, their release depends on the degradation of the ECM proteins, or glycosaminoglycan components, by proteases and the HS-degrading enzyme heparanase, respectively. This degradation induces local release of soluble growth factors from their insoluble anchorage.[17] For example, FGF-2, FGF-10, and VEGFs can be released from perlecan HS, a type of HSPG, by the enzyme heparanase and act as soluble ligands.[109,110] A well-known family of proteases, central to ECM homeostasis, is the matrix metalloproteinases (MMPs), which degrade and remodel matrix macromolecules in their pathway during processes such as cell migration, wound healing, embryonic development, and angiogenesis and therefore play an important role in the release of a variety of growth factors.[111] It is also noteworthy that not all growth factors need to be released from the ECM to bind to their target receptors. It is well established that HSPGs present FGFs to their receptors, using HS as a cofactor.[17]

Alternatively, there are suggestions that ECM proteins present intrinsic growth factor-like domains, which can act as secondary ligands for growth factor receptors. Many ECM proteins, such as laminin, tenascin, thrombospondin, and fibrillin, have small sequences of peptides that are directly recognized by growth factor receptors as soluble ligands initiating GFR signaling.[112–114] Similar to the release of matrix-bound growth factors, the cleavage and release of these peptide segments are mediated by proteolytic degradation. As an example, laminin has EGF like repeats that interact with epidermal growth factor receptor (EGFR) after their release by MMP-mediated proteolysis.[113] In other cases, cleavage is not necessary and the GF-like repeats bind the target receptor in the context of the full-length protein, such as in the case of tenascin C repeats binding to EGFR.[112] In summary, with bound or intrinsic GFs, the ECM regulates cell signaling by acting as a reservoir of GFs and contributing to the establishment of stable gradients, controlling the release of soluble factors and the presentation of solid-phase molecules to bind their receptors.

Once the GF has bound to its specific transmembrane receptor, the GFR phosphorylates tyrosine residues on a number of intracellular signaling molecules, transmitting signals from its position in the cell membrane, but the GF-GFR complex may also be internalized. This will depend on how the GF is presented by the ECM, i.e., binding of a soluble GF might lead to endocytosis and signal modulation, whereas internalization of the GF-receptor complex is more difficult when GFs are presented bound to the ECM.[115] Binding of the GF to its specific receptor initiates a signal transduction cascade, with phosphorylation of the intracellular domains activating kinases and triggering downstream signaling, which eventually results in a particular cellular response (Fig. 4).

B. GFs and integrins signaling pathways cross talk

The coexistence of binding sites for both cell adhesion receptors and growth factors in many ECM proteins allow for local concentration of growth factors near the cell surface. This arrangement yields a specialized biological response by stimulating integrin mechanoreceptor and growth factor receptor signaling in a confined space. It may not always require spatial coexistence, but integrin and GFR co-localization facilitates the cross talk between them.[116] For the past 20 years, intensive research efforts have explained some of the many mechanisms by which the two receptor systems interact.[4,117,118] Given the vast diversity of integrins, matrix ligands, GF receptors, and growth factors, signal integration

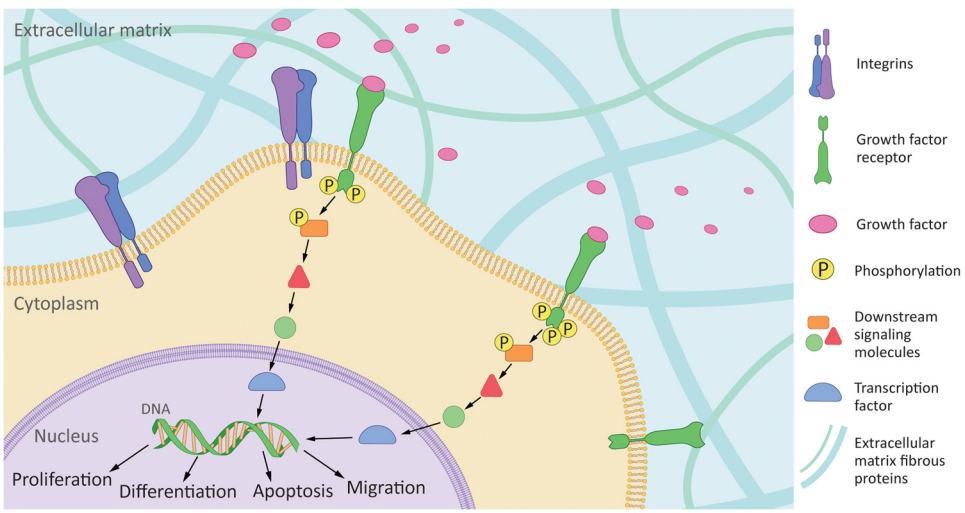

FIG. 4. Illustration of signal transduction cascade initiated by growth factor binding to its growth factor receptor.

between the two types of receptors occur in many different ways and at many different levels. To simplify, these can be organized in five distinct classes of signal interactions between integrins and growth factor receptors (GFRs), namely, collaborative, concomitant, direct activation, inhibitory, and force-dependent signaling, as outlined in Fig. 5 and in Secs. III B 1–III B 5. This division into groups of signal interaction was previously suggested by Ivaska and Heino,[4] with the addition here of the force-dependent signaling as described by Wipff and Hinz[119] and Wipff *et al.*[120]

1. Collaborative signaling

Collaborative associations between integrins and GFRs occur when the natural function of one receptor type results in positive or enhanced signaling of the other receptor type, provided that a GF binds to its receptor. The interplay between the two classes of receptors is bilateral, with GFR activation and signal amplification depending on GFs binding and on cell adhesion to the ECM to create a favorable environment for their interactions with downstream signaling molecules, while the GFR signaling is also essential to regulate integrin expression, stimulate cell adhesion, migration, and other integrin-dependent activities.[4,117,121–124] VEGFR and integrin αvβ3 are good examples of collaborative signaling. The activation of VEGFR is heavily influenced by integrin activation. Specifically, the vitronectin receptor, integrin $\alpha_v\beta_3$, is important in enhancing the VEGF-induced signaling of VEGF receptors leading to angiogenesis.[125,126] The $\alpha_v\beta_3$ integrin has also been shown to have potentiating effects on signaling through several other growth factor receptors, such as the receptors for TGFβ, PDGF and insulin-like growth factor (IGF).[123,127] Other example of collaborative signaling is that of FGF-2 (fibroblast GF-2), which increases the expression of $\alpha_5\beta_1$ integrin in endothelial cells.[124] TGFβ has also been found to increase expression levels of several integrins in keratinocytes, with effects on cell migration and wound healing.[128]

2. Concomitant signaling

Some integrins and growth factor receptors independently activate signaling pathways that can eventually overlap, triggering the same signaling molecules. There are numerous examples of such synergistic signaling between the two types of receptors. One particularly important point of convergence between the two signaling pathways is the protein FAK, which can be either directly phosphorylated by GFR or indirectly activated via Src, acting as a receptor-proximal bridge connecting GFR and integrin.[129,130] The signals from focal adhesions can thus be integrated with that of growth factors and be transmitted through the same pathway (Ras–MEK–MAPK),[131] leading to both modulation of focal adhesion dynamics and cellular functions. Other examples of pathways that are activated by both receptors include the phosphoinositide 3-kinase-Akt pathway and regulation of Rho family GTPases. These shared signaling pathways have been extensively reviewed elsewhere.[132–134]

3. Direct activation of signaling

There are cases of direct growth factor receptor activation by integrin juxtaposition even in the absence of growth factor stimulation. This synergistic event promotes the activation of individual pathways in an integrin specific manner.

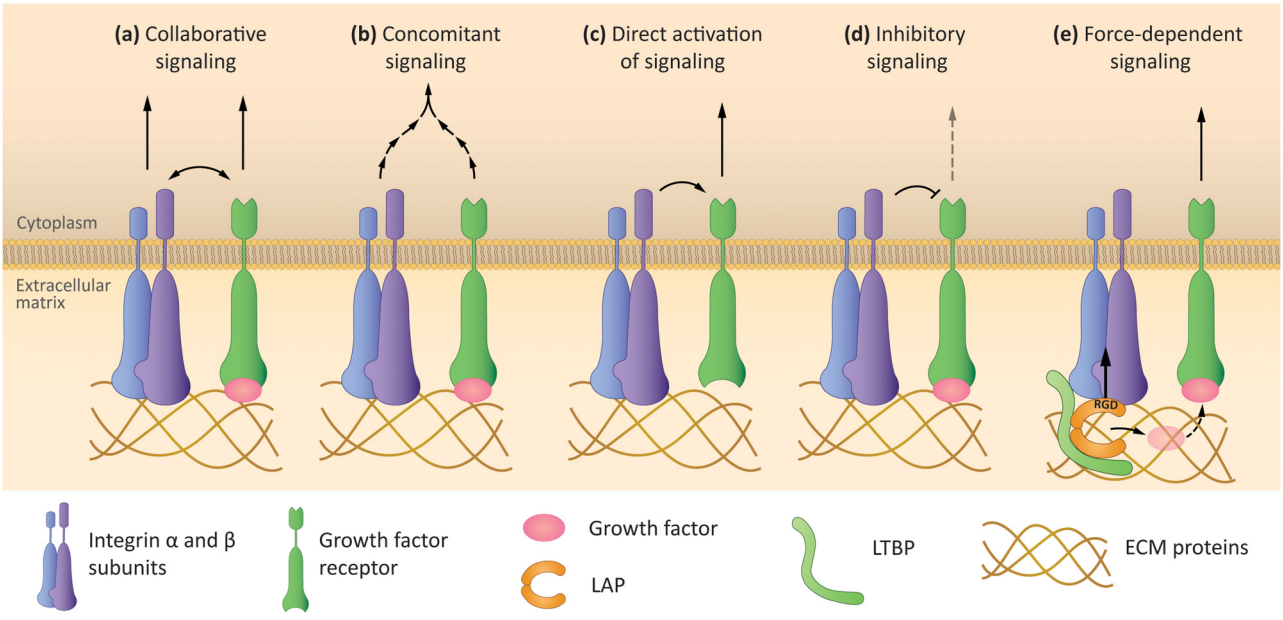

FIG. 5. Distinct mechanisms of activation of cellular signaling pathways between integrins and GFRs: (a) In collaborative signaling, integrin enhances GFR signaling and vice versa. (b) In concomitant signaling, integrin and GFR independently activate the same pathway. (c) Direct activation happens when GFR is activated by integrin juxtaposition in the absence of GF. (d) Inhibitory signaling refers to the negative regulation of GFR signaling caused by integrin-ECM interaction. (e) In force-dependent signaling, contractile activity transmitted through integrins on the LLC leads to release of active TGF-β1.

For example, mesenchymal stem cell (MSC) adhesion to fibronectin through $\alpha_5\beta_1$-integrin has been found to induce PDGFR-β phosphorylation in the absence of growth factor stimulation.[135] Immunofluorescence microscopy revealed that phosphorylated PDGFR-β co-localized with $\alpha_5\beta_1$ at focal adhesions. This synergistic interaction between $\alpha_5\beta_1$-integrin and PDGFR-β aligned to a fibronectin-rich matrix appears to be fundamental in mesenchymal cell migration to sites of vascular remodeling.[135] Another example of such direct activation can be found in integrin-induced EGF receptor activation, which leads to phosphorylation of EGF receptor tyrosines.[136] It must be pointed out that the sites to be phosphorylated on the GFR through this intriguing mechanism of integrin-induced GFR activation are distinct from those triggered by GF stimulation alone. For instance, $\alpha_v\beta_3$ integrin-dependent adhesion induces phosphorylation of specific tyrosine residues on the EGF receptor, distinct from those induced by the soluble ligand EGF,[136] and therefore leading to altered signaling and biological responses.

4. Inhibitory signaling

Although in most cases integrins appear to function as positive regulators of GFR signaling, cell-matrix interactions may also generate GF signaling inhibition. An example of this was found when studying the binding of $\alpha_1\beta_1$ integrin to collagen type I in HeLa cells. This was seen to result in membrane recruitment and activation of T-cell protein tyrosine phosphatase (TCPTP).[137] The activation of TCPTP thereafter induced dephosphorylation of EGFR, VEGFR2, and PDGFRβ, and consequently attenuation of their signaling.[137,138] Interestingly, it seems that both integrin-induced activation in the absence of GF and negative regulation of GFR phosphorylation direct the signaling into given downstream pathways other than those normally triggered by the GFRs.[4]

5. Force-dependent signaling

Another, quite different, yet relevant mechanism of GFR activation involves the physical coupling of integrins to the ECM. This was exemplified in a study by Wipff *et al.*[120] where a model of an *in vitro* scar was used to show that the differentiation process of fibroblasts into myofibroblasts depended on both the presence of mechanical stress and active TGF-β1. Secreted TGF-β is presented in the ECM as inactive proteins noncovalently bound to its propeptide (latency-associated propeptide—LAP), which contains binding sequences for various integrins (i.e., RGD). The TGF-β/LAP complex is linked to an additional protein, latent TGF-β binding protein (LTBP), forming the large latent complex (LLC). In most cells, TGF-β is secreted as LLCs and, in the ECM-bound state, TGF-β is therefore inaccessible to their transmembrane receptors.[139] The growth factor release from the ECM depends on binding of integrins to the RGD motif in the propeptide and cell contraction by the actin cytoskeleton, inducing conformational change of the LLC and releasing the active TGF-β[119,120,140] [Fig. 5(e)]. Furthermore, the small GTPase RhoA has been shown to mediate TGF-β release from its inactive secreted complex, through its well-established roles in contractility of the intracellular cytoskeleton.[141] Thus, it should be emphasized that the efficiency of latent TGF-β signaling is affected by the composition and rigidity of the ECM, and by cell contractile forces during cell motility.[100]

To summarize Sec. III, growth factors are soluble secreted proteins facilitating cell-cell and ECM-cell communication. GFs bind to transmembrane receptors and initiate signal transduction leading to a raft of biological responses including cell cycle control, differentiation, and tissue homeostasis. GFs are found bound to the ECM, and the ECM is central in regulating the availability and activity of GFs *in vivo*, for example, by proteolytic ECM degradation, commonly in proximity to matrix adhesions, leading to GF release. Integrins and GF receptors often co-localize in the cell membrane, facilitating cross talk between them. Such cross talk can occur in many different ways, such as mutual activation enhancement, shared signaling pathways and through mechanically induced release of GFs at focal adhesions, able to act on co-localized GFRs.

IV. ENGINEERED BIOINTERFACES DEVELOPED TO STUDY MECHANOTRANSDUCTION

Engineered biointerfaces have been developed to study the influence of properties such as the presentation of biochemical signals, surrounding topography and elasticity on the cell response. The following sections present a variety of approaches to mimic physical properties of the ECM. These studies helped both to obtain a more comprehensive understanding of mechanotransduction and demonstrated the potential for applications in the field of regenerative medicine.

A. Spatial distribution of biochemical signals

Biochemical signals in the form of cell-adhesive ligands are presented *in vivo* at various size scales and geometries. The relevant biochemical signals considered here are integrin ligands, either as full-length ECM proteins (laminin, collagen, vitronectin, fibronectin, etc.) or as synthetic peptides derived from these proteins (such as RGD—both linear and cyclic versions, IKVAV and YIGSR) often used as signals presented from surfaces.[142] For the interested reader, immobilization strategies for growth factors and other proteins have been reviewed thoroughly elsewhere.[143–145] The ability to make surfaces presenting such ligands at a small feature size is one key component in order to study the details of cell surface interactions. Mammalian cells differ in size but are typically in the range of tens to hundreds of micrometers, and the influence of micropatterns on cells have been extensively studied.[146–148] As the focal adhesions anchoring cell surfaces are in the low micrometer range and the integrins are in the nanometer range (see Fig. 1), there has also been an increasing interest in studying how nanometer size features influence cell behavior. Adhesive patches on a nonadhesive background

can be used to define areas of cell growth limited to few or single cells. This is useful for the organization of cells in applications of cell based sensing or drug discovery.[149,150]

Pioneering work by Chen *et al.*[15] on the restriction of cells on small adhesive micropatterned areas revealed that cell shape influences cell survival. It was seen that an increasing number of cells entered apoptosis as the adhesive patch decreased to circles with a diameter of 20 μm. Micropatterns of cell-adhesive regions have been shown to influence not just cell shape and survival but also adhesion strength,[151,152] intracellular organization[146] and polarity,[153–155] gene expression,[156,157] migration,[158,159] and differentiation.[160,161]

The submicrometer length scale is interesting as it is the length scale of focal adhesions. Culturing cells on patterns of adhesion molecules at this scale is expected to have a strong effect on cell adhesion and focal adhesion formation. Lehnert *et al.*[162] explored the geometrical limits of cell adhesion, spreading, and migration by employing microcontact printing to create individual arrangements of square dots with varying sizes and distances. They found that cells could not spread on patches smaller than 0.1 μm^2 if spaced 5 μm apart. Patterns of varied geometries suggest a lower limit of adhesive ligands required for focal adhesion formation.[163,164] In a later study, Arnold *et al.*[165] used biofunctionalized micro-nanopatterned surfaces to determine the minimum number of activated integrins necessary to induce the formation of focal adhesions [Fig. 6(a)]. They created hierarchical patterns of RGD functionalized gold nanoparticles with side lengths ranging from 100 to 3000 nm where cells showed a tendency to bridge between adjacent domains for patterns below 500 nm in order to adhere. Aiming to investigate the minimal size of a focal adhesion that supports cell adhesion and/or spreading, Malmström *et al.* produced submicrometer protein patterns over large area using colloidal lithography[13,166–169] [Fig. 6(b)]. They found that cells could adhere and spread on adhesive patches as small as 200–300 nm in diameter. It is believed that this is approaching the smallest geometrical size of an integrin cluster that can lead to a mature (connected to actin fibers) focal adhesion—a process controlled by the force exerted on the actin network by the focal complex.[3,170] By comparing such small patches of two different ECM proteins, vitronectin and fibronectin, it was also found that the type of protein displayed changed the mechanotransduction. Single focal adhesions were observed to bridge across gaps between ligand patches only on the intracellular size. Such bridging was seen for vitronectin patches of 0.2 μm but not until 0.5 μm for fibronectin. This was interpreted as being due to fibronectin being able to modulate the force exerted at these small patch sizes by unfolding.[169] In a follow-up study using the same pattern fabrication approach, Gautrot *et al.*[6] demonstrated that epidermal stem cell spreading and differentiation depended on such spatial organization of adhesions sites. Interestingly, by simply restricting the ECM protein into small patterns, a very similar behavior was seen as for cells cultured on very soft substrates.[9] This was attributed to the restricted amount of force that could be transduced through such a small adhesion site. The authors also investigated if the effect was a result of biochemical maturation of the adhesion, but found that while the nanoscale frustration of adhesions regulated the mechanotransduction, this was independent of FA-protein recruitment, phosphorylation, and matrix deposition.[6]

At the size range of single integrins, the precise location of synthetic $\alpha_v\beta_3$ integrin ligands (cRGDfK) spaced by an antifouling background was achieved by Arnold *et al.*[14] The peptides were coupled to gold nanodots deposited in an ordered array using block copolymer micelle nanolithography,[171,172] where the spacing between the gold dots was controlled by the polymer chain length.[171,173–175] By creating such patterns, it was demonstrated that the range between 58 and 73 nm could be regarded as a universal length scale for the clustering of integrins on 2D flat surfaces in a variety of cell types. These findings led to a step-change in the understanding and research on the importance of the spatial distribution of cell adhesion ligands. This approach has also been extended to full-length protein patterns, hierarchical patterns,[165,176] and flexible substrates.[177] These studies have helped to elucidate how the ligand presentation affects cell morphology, survival, attachment, and migration and further provided knowledge about how researchers can exploit these systems to direct cell fate.

Block copolymers have been used in several other approaches to pattern adhesion peptides to understand cell behavior.[178–182] For example, polystyrene-*block*-polyethylene oxide (PS-*b*-PEO) substrates were created resulting in a hexagonally arrayed PEO nanocylinder domains with a diameter of ~17 nm and a lateral spacing of ~35 nm.[183] By blending a functionalized and a pristine PS-*b*-PEO, it was possible to control the density of RGD or IKVAV ligands [Fig. 6(c)] presented at the PEO domains. The authors showed that the morphology, cytoskeletal actin stress fibers, and focal adhesion maturation in mesenchymal stem cells were significantly affected by the ligand presentation. This flexible and straightforward approach offers a platform to study the impacts of ligand redundancy and affinity on surface-mediated regulation of hMSC feedback.[184] However, with distances between neighboring peptide patches being below the critical integrin spacing identified previously, the biological effects in these studies are more subtle.[183,184] It is also important to note that in this approach the surrounding substrate area is not protein rejecting as in the block copolymer micelle approach discussed previously. Thus, performing experiments in serum-free media is of paramount importance. In a similar approach, Frith *et al.*[185] achieved a lateral spacing of RGD peptides by coupling maleimide functionalized PS-*b*-PEO to cysteine-GRDGS. Blending this compound with PS homopolymers at different block copolymer/homopolymer ratios led to a lateral cell adhesion spacing from 34 to 62 nm. When culturing hMSC on these substrates, it was evident that these cells adhered specifically to RGD moieties via α_5, α_V, β_1, β_3, and β_5 integrins [Fig. 6(d)]. Furthermore, it was shown that with increasing lateral spacing the MSC spreading and osteogenesis were reducing while adipogenesis was promoted. These findings demonstrate how lateral spacing of

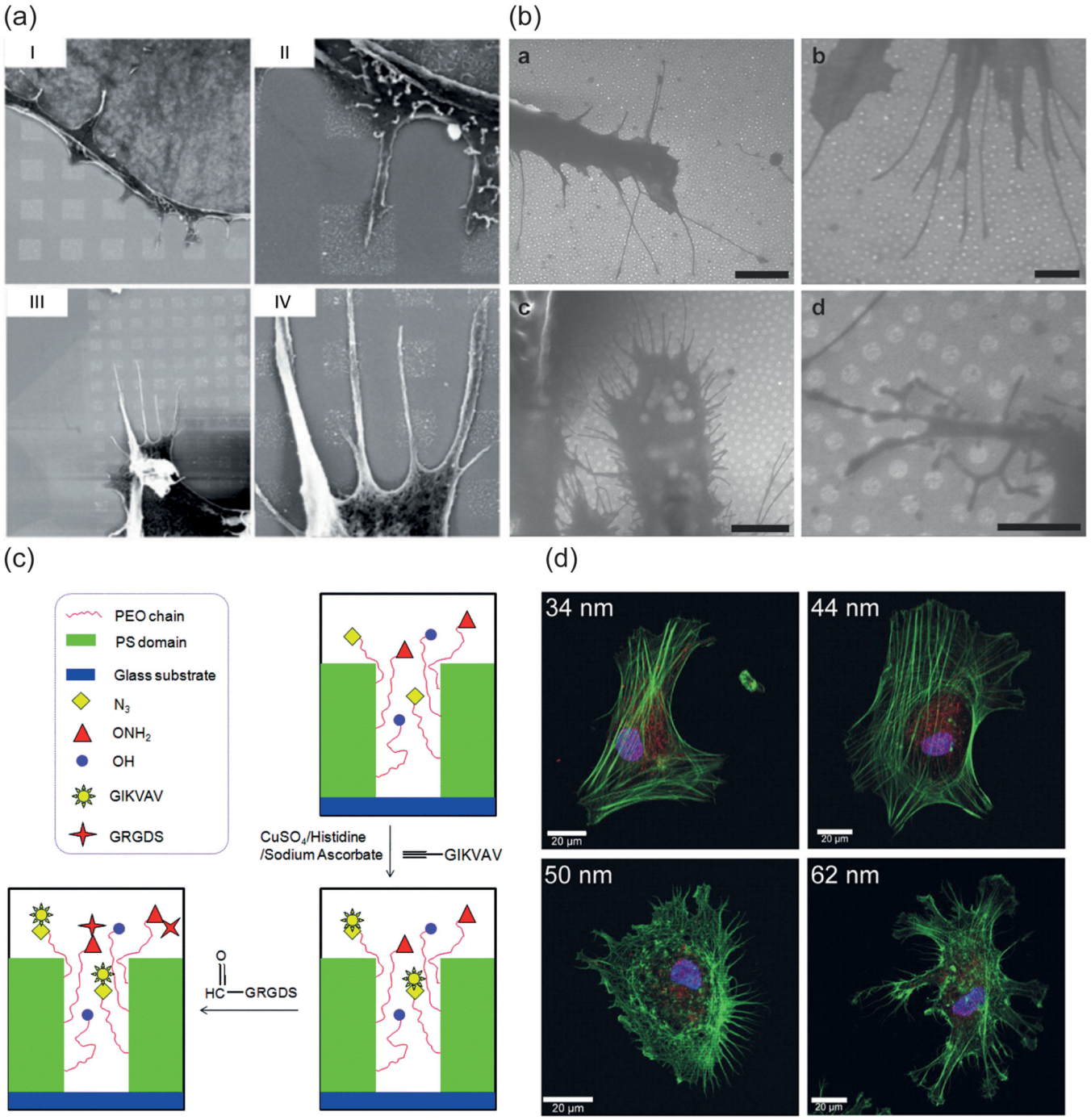

FIG. 6. Spatial distribution of ligands in the micro- as well as in the nanoscale can significantly alter the cell response. (a) On patterns with a square size of 1 μm (I, II), FA size is restricted by the underlying geometry. In contrast, on patterns with a square size of 500 nm (III, VI), adjacent paxillin domains are bridged by an overlying actin fiber. Reproduced figure from Arnold *et al.*, Soft Matter **5**, 72 (2009). Copyright 2009, The Royal Society of Chemistry. (b) SEM images of cells adhering to FN adsorbed on 200 nm protein patches in (a) and (b) and 500 nm protein patches in (c) and (d) (scale bar 2 μm). Reprinted with permission from Malmström *et al.*, Nano Lett. **10**, 686 (2010). Copyright 2010, American Chemical Society. (c) Sketch of the stepwise functionalization of PS-*b*-PEO thin films with IKVAV and RGD. Reproduced figure from Li and Cooper-White, Biomater. Sci. **2**, 1693 (2014). Copyright 2014, The Royal Society of Chemistry. (d) Lateral spacing (34–62 nm) of RGD peptides on 2D substrates influences hMSC morphology and cytoskeleton organization. Reproduced figure with permission from Frith *et al.*, J. Cell Sci. **125**, 317 (2012). Copyright 2012, Company of Biologists Ltd.

adhesion peptides in the nanoscale can influence stem cell behavior, which can be explained by the inhibition of integrin clustering at increased RGD spacing.

PS-*b*-PEO has also been used to create hierarchical patterns by Tran *et al.*[186] Combining the bottom-up self-assembly abilities of block copolymers with photolithography allowed for the fabrication of sub-50 nm hierarchical patterns of biomolecules. Due to the precise spatial control, these biomolecule patterns enable detailed studies of cell adhesion, spreading, migration, and differentiation.

In addition to the application as templates for substrate fabrication methods, block copolymers have also been used to arrange proteins into higher order structures in solid state films. The most prominent approaches describe the co-assembly of block copolymers and proteins and the use of peptides or proteins as one block of the polymer,[187] while proteins and peptides have also successfully been included into films noncovalently[188–191]—an approach with potential drug-delivery benefits. Overall, combining the adjustable morphology, the ability of hosting and release active biomolecules and the possibility to introduce a variety of functional groups make block copolymers a versatile tool for cell studies. However, the use of thin films with thicknesses of less than 100 nm for cell culture comes with some challenges. The prolonged exposure to cell media can lead to film dewetting, structural rearrangement, or topographic reconstruction.[192–194]

B. Topography

Another important factor that influences cell behavior is the surrounding topography.[195–197] Cells are able to respond to topographical features on surfaces with an impressive sensitivity *in vitro* of down to 5 nm.[198] Topographical features can generally be summarized into surface roughness and surface patterns, divided into isotropic and anisotropic patterns.[195,199] Methods to induce surface roughness such as etching or sand blasting[200,201] are very simple compared to elaborate patterning methods. Yet, surface roughness has been proven to have a significant effect on cell adhesion independent of the cell type and substrate materials.[202–206] The *in vitro* results for roughness give less clear results than those from controlled geometries such as ridges and grooves, but a trend seen is that adhesion, migration, and ECM production increase with increased roughness.[207] Considering the influence of nanoscale topographical features on cells, it is notable that nanotopography has been shown to influence the adsorbed protein layers, which in turn can affect the cell response.[208–210] While we will not discuss the influence of surface roughness on cell fate in great detail, it is worth mentioning that Lyu *et al.*[211] studied the regulation of behavior of mouse embryonic stem cells (mESCs) on gold nanoparticle layers with nano-, submicro-, and microscale surface roughnesses. They found that a roughness (root-mean-square average, R_q) greater than 573 nm led to a significant loss of mESC pluripotency, while roughness below 392 nm resulted in long-term maintenance of mESC pluripotency.

The response of cells to ridges and grooves is more pronounced than the response to roughness. Cells generally respond to such features in the micrometer range by aligning along the structures—a phenomenon called contact guidance.[212–215] The response is dependent on both groove width (cells responding to features from the size range of cells and down) and depth (orientation increasing with increased groove depth) and has perhaps not surprisingly been seen to be cell type dependent.[213,216,217] Other effects include differences in cytoskeletal organization, higher motility, and influences on adhesion and differentiation.[213,218,219] The results have been more varied at the smaller scale. Grooves in the nanometer range have been shown to lead to alignment of many, but not all, cell types *in vitro*.[220–223] A recurring trend is that alignment of cells increases with increasing groove depth.[224] In one study, Teixeira *et al.*[225] examined the relevance of nanotopography for human corneal epithelial cells. To do so, they lithographically fabricated substrates with a parallel groove topography and size features from 20 to 200 nm. In contrary to smooth substrates, which mainly lead to round cells, cells stretched along the ridges of grooves as small as 70 nm [Fig. 7(a)]. They could further observe that the width of the focal adhesions corresponded to the width of the features, and that the focal adhesions were aligned along the grooves. For pores and pillars in the micrometer range, cells have been observed to engulf pillars or to stretch between adjacent pillars and to attach preferably to the edge of pores.[226] The results of Teo *et al.*[227] contribute to the understanding of topography-induced differentiation mechanism in hMSCs. Using a fibronectin-coated nanograting, fabricated by nanoimprint lithography, the authors could demonstrate that the focal adhesion characteristics of human stem cells were modulated by the dimension of the topography [Fig. 7(b)]. In addition, they identified the FAK as a vital early mechanosensor of ECM topography. Furthermore, their findings suggested that neuronal lineage differentiation of hMSCs was mainly a function of the spatial and temporal regulation of FAK phosphorylation. In an attempt to mimic the properties of the ECM *in vitro* more precisely, Das *et al.*[228] created networks of synthetic twisted and helical fibrils with nanometric periodicities on glass substrates. The results from the analysis of hMSC response indicated that the stem cells were able to interpret the nanohelical shape and periodicity, where helical nanoribbons of a specific periodicity of 60 nm promoted the formation of focal adhesions as well as the differentiation into an osteoblast lineage. In contrary, twisted ribbons with a periodicity of 100 nm did not lead to osteoblast commitment. This outcome represents another approach to control cell differentiation *in vitro* via surface cues, and this particular approach is interesting for being potentially exploitable for 3D scaffolds aimed at tissue engineering applications.

C. Stiffness

Reports that cells can sense the mechanical properties of their microenvironment and deform inert materials date back to the 1980s. Early studies observed how cells could wrinkle soft silicone substrates.[229] Substrate stiffness, as a physical factor influencing the stem cell fate, was identified as crucial for cell migration,[230] proliferation,[231] spreading,[232,233] and differentiation.[234–236] A key method for the study of the influence of different substrate stiffnesses on cell behavior is the use of hydrogels.

In a pioneering study, Engler *et al.*[10] explored the previously undocumented influence of substrate stiffness on MSC fate by analyzing the response of cells on polyacrylamide (PAA) hydrogels with different degrees of cross-linking. As

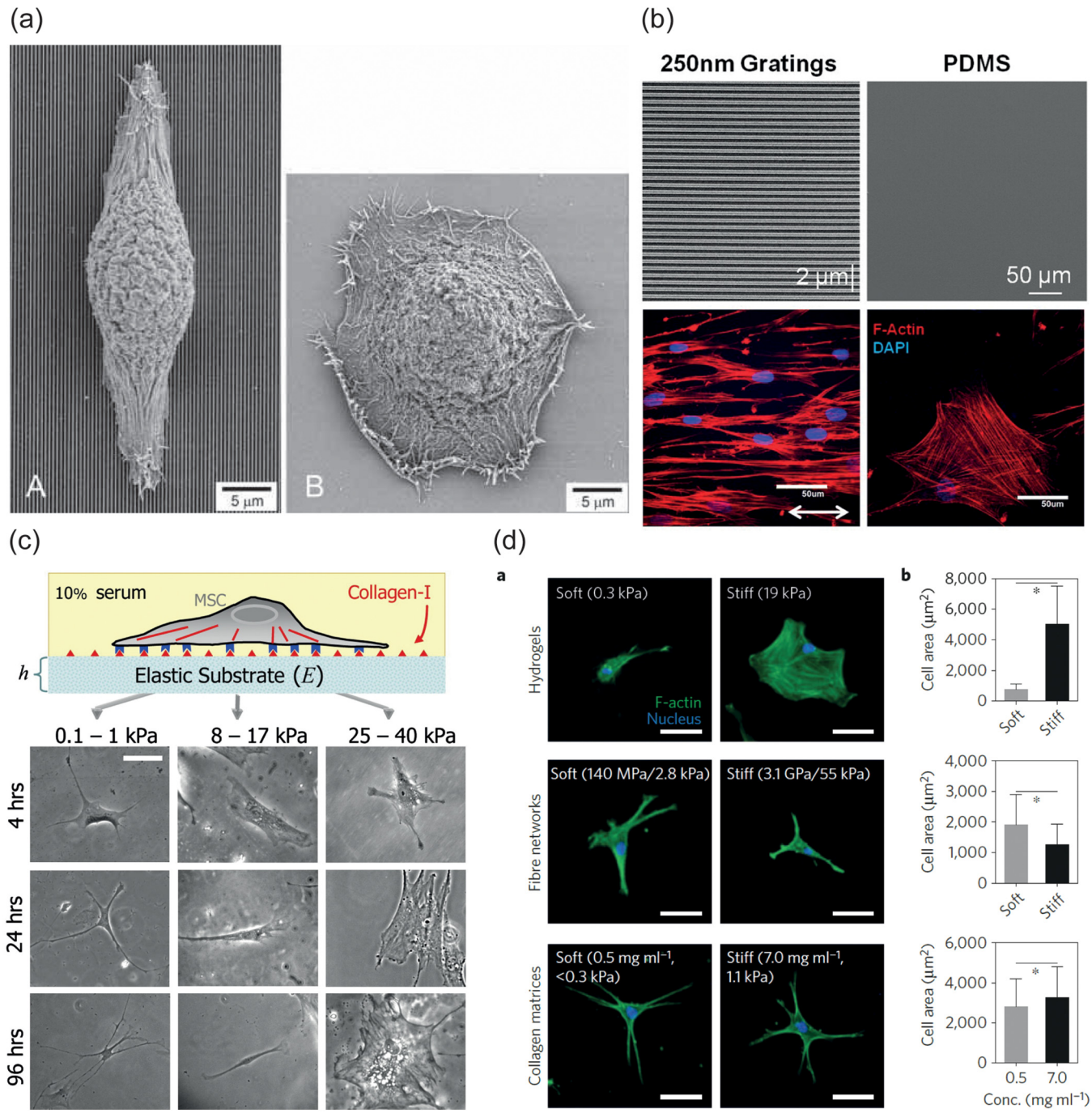

FIG. 7. Topography as well as the substrate stiffness can affect the cell morphology and spreading of various cell types. (a) SEM image of HCEpC cells cultured on silicon oxide patterns with 70 nm wide and 600 nm deep ridges (left). HCEpC cells on a smooth silicon oxide substrate (right). Reproduced figure from Teixeira *et al.*, J. Cell Sci. **116**, 1881 (2003). Copyright 2003, Company of Biologists Ltd. (b) SEM images of nanogratings and unpatterned substrates (top). Fluorescently labeled hMSCs on corresponding substrates, clearly showing cells aligning along the gratings. Actin is stained red and cell nucleus blue. Reprinted with permission from Teo *et al.*, ACS Nano **7**, 4785 (2013). Copyright 2013, American Chemical Society. (c) Sketch of *in vitro* system depicting an MSC growing on a collagen-I coated elastic substrate (top) and microscopy (bottom) showing the morphological changes of MSCs as a function of the stiffness range and the time of cell culture. Reprinted with permission from Engler *et al.*, Cell **126**, 677 (2006). Copyright 2006, Elsevier. (d) Actin cytoskeleton (green) and nucleus (blue) of hMSCs on hydrogels, fiber networks and collagen matrices of different elasticities and the corresponding cell areas (right)—highlighting the importance of fiber recruitment. Reprinted with permission from Baker *et al.*, Nat. Mater. **14**, 1262 (2015). Copyright 2015, Springer Nature.

depicted in Fig. 7(c), collagen-coated PAA gels were fabricated in such a way that their elasticity was in the range of brain, muscle, and collagenous bone tissue, respectively. Here, it was evident that the matrix stiffness drastically altered the differentiation of MSCs promoting neurogenic (0.1–1 kPa),[237] myogenic (8–17 kPa),[238] and osteogenic (25–40 kPa)[239,240] differentiation. The majority of the studies were focusing on hydrogels with varying bulk stiffnesses on

flat surfaces. In a major study, Trappmann *et al.*[9] studied the human epidermal stem cell fate on biofunctionalized hydrogels of varying stiffnesses. These polydimethylsiloxane and polyacrylamide gels were covalently cross-linked with collagen. Interestingly, they could conclude that not solely the difference in stiffness leads to an altered cell response but the density of anchor points contributed. Their findings demonstrated that the relationship between the substrate stiffness and cell behavior is translated through the mechanical feedback of the ECM. Thus, the stem cell response is dependent on the mechanical force applied on substrate-bound ECM, which exhibits a previously unidentified stem cell-niche interaction. Winer *et al.*,[241] however, could show that fibroblasts and hMSCs adopted a stretched morphology when they were cultured on two-dimensional fibrin gels. They found that a variety of cells were able to deform the substrate, which locally and globally stiffened the gel and consequently altered cell-cell communication and alignment. These results highlight the impact of both viscous and elastic properties of the material on cell response.[241] Such stress-stiffening behavior and its influence on stem cell fate have also been shown in 3D. By encapsulating hMSCs in polyisocyanopeptide-based gels of constant stiffnesses but varying critical stress values for the onset of stress stiffening, it was possible to switch hMSC fate from adipogenesis to osteogenesis.[242] Thus, the differentiation was modulated without changing the bulk stiffness of the gel, while mimicking the stiffness of adult stem cell niches from 0.2 to 0.4 kPa.[242] While the majority of studies on the influence of mechanical properties have focused on model systems using flat hydrogel substrates of varying stiffnesses, Baker *et al.*[243] developed a system composed of synthetic fibers with tuneable mechanic properties and studied the effects on cell spreading and proliferation [Fig. 7(d)]. In contrary to commonly used flat hydrogels, their system takes the composition of the ECM into account, which can more precisely be described as a meshwork of fibers with diameters in the micrometer range.[244,245] Analysis of the hMSC responses on electrospun DexMA (Ref. 246) fibers revealed that multiple structural parameters such as fiber stiffness, density, and fiber-fiber welding influenced the cell function. Growing in lower stiffness regimes enabled cells to rearrange adjacent fibers, which resulted in increased local adhesive ligand density, cell spreading, and proliferation signaling. In summary, this suggests that spatial rearrangement in the ECM can also provide feedback to alter cell function and signaling.[247]

In contrary to the previously mentioned studies, Kong *et al.*[248] studied the cell adhesion and proliferation of HaCaT cells at the surface of low viscosity liquids, where the surface was stabilized in-plane by self-assembled protein sheets. Prior to their study, only a few reports have demonstrated cell culture on liquid substrates due to fast relaxation times of liquids, which do not allow the stabilization of focal adhesions.[249–252] In their work, they showed that cell proliferation as well as culture could be performed effectively on liquid-liquid interfaces regardless of the lack of a solid substrate. These findings can be explained as the self-assembled protein nanosheets allowed force transduction in-plane, but not out-of-plane. These findings suggest that bulk and nanoscale mechanical properties can be designed independently to design suitable biomaterials for regenerative medicine.[248] This discovery potentially enables applications in other platforms such as microdroplet systems or the design of biomaterials and implants.[253] In the field of biomaterials and tissue engineering, it is therefore important to keep the mechanical properties of the host tissue in mind during design. It has even been suggested that cells exhibit a mechanical memory that is able to store information from past physical environments.[254,255] The temporal role in cellular mechanotransduction potentially has a significant impact on both fundamental understanding of stem cells and their use in tissue engineering.

The term durotaxis describes the phenomena of cells migrating along stiffness gradients. For example, human adipose-derived stem cells (hASCs) have been observed to migrate toward higher stiffness,[256,257] while neurons migrate toward softer substrates.[258] It has been found that the cell velocity generally correlates to the gradient strength.[257] In a straight forward, yet efficient approach, Hadden *et al.*[259] developed a method to produce planar polyacrylamide hydrogels with a linear stiffness gradient. Studying the cell migration of hASCs on different stiffness gradients revealed a narrower durotactic threshold than previously reported.[256,257] These findings are particularly interesting for two reasons. Firstly, gels with a stiffness gradient below the upper threshold of 8.2 kPa/mm can be used to study cell migration toward stiffer and softer areas. Secondly, gels with stiffness gradients below the durotactic threshold of 2.9 kPa/mm can be used to analyze cell behavior on a wide range of stiffnesses perceived by the cells as constant. Therefore, a system like this, fabricated with only a few components, further offers the opportunity to study of a wide range of biological conditions or disease processes.

To summarize Sec. IV, many different engineered model systems have been developed to study mechanotransduction. It is interesting to compare the approaches of material stiffness modulation with precisely located integrin ligands, and it has been shown that similar biological responses can be found when comparing low stiffness materials with materials restricting the geometrical maturation of focal adhesions. Simple topographic patterns can also initiate similar biological responses, depending on the size and spacing of such patterns. However, various topographies can also modulate a raft of other biological responses through, for example, physically aligning cells, or disturbing processes such as cell division or migration. While care has to be taken before generalizing results seen in well-defined model systems, a lot of detailed knowledge has sprung out of these engineered systems, which would not otherwise have been attainable.

V. ENGINEERED BIOINTERFACES TO STUDY GF SIGNALING

In addition to the biophysical signaling, described above, cells also respond to biochemical signals such as growth

factors, hormones, or cytokines. In this review, we want to outline the interaction between growth factor signaling and mechanotransduction in particular. As outlined in Sec. III, GFs are significant biological signals playing a role in cell growth, stem cell differentiation, and proliferation.[260] Many methods for GF delivery (both *in vivo* and *in vitro*) developed to date rely on supplying soluble GFs. This approach is known to lead to dosage problems, providing either an inefficient dose, or, in some cases, causing side effects due to the supra-physiological doses that are required due to the low stability of growth factors.[261–263] For example, biomaterial carriers have been used *in vivo*, to release growth factors, such as the release of bone morphogenic protein from collagen sponges in spinal fusion applications.[264,265] The potency of these molecules has the potential to lead to significant side effects, however,[96,261,262,266] why the mode of delivery and local concentrations needs to be tightly controlled to avoid off-target effects. Adding a solution of growth factors to the cells growth media is not representative of the presentation of GFs *in vivo*, where GFs are bound to and regulated by the ECM.[267–269] Furthermore, GFs have rather short half-lives and depict toxicity at high doses.[270] Thus, engineered biointerfaces aim for a concentration-controlled and a spatiotemporal-controlled presentation of GFs.[96,271] Growth factors can either be physically or covalently attached to a surface or released from a material.[272] In one pioneering study, Kuhl and Griffith-Cima[273] demonstrated the tethering of EGF to a functionalized glass substrate via star-shaped poly(ethylene oxide). The EGF was found to retain its biological activity and was shown to be as effective as soluble EGF. When the authors simply physisorbed the EGF, it did however not exhibit biological activity. One simple way to incorporate growth factors in materials is to encapsulate GFs into 3D gels. For example, Murphy *et al.*[274] demonstrated the encapsulation of VEGF into poly(lactic-co-glycolic acid) gels and showed that the activity of released VEGF was 70% after 12 days of storage in simulated body fluid and phosphate-buffered saline solutions. Contrary to covalently bound growth factors, the physical adsorption or incorporation of growth factors can be achieved by simply mixing growth factors and polymers before the gelation process or adsorption on surfaces. However, the presentation of physically adsorbed growth factors comes with drawbacks such as poor stability and uncontrolled release effects.[270,275] Multilayer systems have been suggested for GF storage and release, as they may allow for spatial and temporal control of GF release.[276,277] Specifically, layer-by-layer (LbL) assembly has been used to deposit oppositely charged polyelectrolytes and GFs into functional polymer coatings on surfaces.[276–283] Recent developments in this area include automated LbL assembly of biomimetic coatings in cell culture microplates for high throughput screening of cell adhesion and differentiation in response to peptides and GFs included in the coatings.[284] LbL assemblies could depict a tuneable release of growth factors as well as only minimal burst release depending on the choice of polyions and the overall charge density of the proteins embedded in the film.[285–287]

Another way to avoid burst release and enhance the stability of growth factors is the covalent immobilization of growth factors.[143] Growth factors that are covalently attached to surfaces or matrices can be regarded as immobilized. The release rate (if applicable) would then be a function of the enzymatic or hydrolytic cleavage rate of the chemical bond. However, growth factors may lose their biological activity because of the damage of active sites in the immobilization reaction, or through steric hindrance of binding to GFRs in the immobilized form. Other methods explored for GF presentation involve an ECM-inspired delivery system, in which GFs were tethered to ECM molecules such as heparin due to their natural bioaffinity.[288,289] One study that has been central to the understanding of the importance of GFR internalization is the study by Schwab *et al.*[290] In this work, they produced surfaces with patterns (interparticle distance of 36 ± 6, 56 ± 8, and 118 ± 18 nm) presenting a controlled surface density of covalently bound BMP-2 dimers achieving a local and sustained presentation to C2C12 myoblasts. Their work demonstrated that BMP-2 in its immobilized form was more efficient than in its soluble form to stimulate the Smad-transcriptional pathway, main signal transducer for receptors of the TGF-β superfamily.[291] In contrary to previous studies, the results also indicated that the ligand in this case did not need to be endocytosed to propagate Smad signaling. The authors further suggest that this method could act as a useful tool to study threshold concentration of growth factors and the minimum amount of growth factor receptors necessary for activating cell responses. In this particular case though, the lowest presentation density of 0.2 ng/cm² was still found to be high enough to trigger signaling.[290] This highlights that these growth factor receptors do not need to cluster (at least not below the threshold examined) to activate signaling, in contrast to the force mediated signaling occurring through integrin complexes.

To summarize Sec. V, several different approaches have been developed to present and deliver GFs to cells from surfaces or gels. Tethering GFs or GF mimetic peptides from surfaces have been successful for prolonged presentation and activity of GFs, and materials such as gels and layer-by-layer assemblies show potential for controlled local delivery of growth factors. Materials allowing spatiotemporal control of the display or release of growth factors are particularly relevant.

VI. ENGINEERED MATERIALS TO STUDY MECHANOTRANSDUCTION AND GF SYNERGIES

Over the past few years, cell biologists and biomaterials scientists have put efforts together to engineer new materials that mimic part of the *in vivo* interactions between cells and their surroundings.[292–295] Inspiration has been drawn from how specific growth factors and integrin-binding sites are presented in the extracellular microenvironment and in the way GFs are presented as soluble or solid-phase molecules to their target cells.[296–298] Engineered bioinspired systems

that enable the simultaneous activation of integrins and GFs offer the possibility to reduce GF doses and increase its signaling efficiency. Well-defined model systems to study mechanotransduction and growth factor signaling together will be needed to understand the subtleties of the synergies between the two.

One strategy being explored for localized presentation of integrin ligands and GFs is to mimic the specific components of the ECM, since many of the ECM proteins naturally bind both growth factors and integrins in a confined space.[5] For example, a number of biomaterials have been modified with heparin or heparan sulphate-mimetic molecules to sequester heparin-binding growth factors and control their release.[299–301] In particular, there has been a significant amount of work using fibronectin fragments to design materials based on their ability to bind other fibronectin molecules, GFs and integrins.[302] In this way, engineered materials using fibronectin have been reported to foster the co-localization and simultaneous activation of integrins and GF receptors, important for stem cell differentiation.[302–304] For example, osteoblastic differentiation was reported for MSCs cultured in a fibrin matrix cross-linked with a multifunctional recombinant fragment of fibronectin containing the major integrin-binding domain ($FNIII_{9\text{-}10}$) and the growth factor-binding domain ($FNIII_{12\text{-}14}$),[302] which promiscuously binds GFs, including VEGF, PDGF, FGF, IGF, and BMP-2.[103] This strategy efficiently improved GF-induced wound healing and bone repair with much lower doses of GFs than the techniques previously reported, although this has only seen when FN $III_{9\text{-}10}$ and FN $III_{12\text{-}14}$ are proximally presented in the same polypeptide chain.[302] Similarly, a biodegradable hyaluronic acid (HA) hydrogel covalently grafted with $\alpha_5\beta_1$ integrin-specific FN fragment ($FNIII_{9\text{-}10}$) was used as a carrier for human bone morphogenetic protein-2 (rhBMP-2), and it was found to enhance the osteogenesis *in vivo*.[304] The authors found that the functionalized HA with FN fragments promoted the formation of twice as much bone compared to when delivering the growth factor in a nonfunctionalized HA hydrogel. This result is likely attributed to better cell adhesion to the hydrogel, as well as due to potential strengthened osteoinductive signaling generated by the cross talk between the integrin and BMP-2 receptor.

Another approach to boost the ability of fibronectin to bind to integrins and GFs simultaneously involves the unfolding and attachment of this protein to the surface of materials. Poly(ethyl acrylate) (PEA) has been shown to trigger fibronectin unfolding and self-organization upon adsorption.[305] It was found that fibronectin adsorbed to PEA facilitated synergistic integrin and GF signaling by promoting high availability of integrin- and GF-binding regions adjacent to each other on the unfolded FN, thus co-localizing integrin (β_1) and the BMP-2 receptor.[305] Specifically, it was found that this material encouraged cell differentiation to osteoblasts through ERK1/2 and Smad signaling *in vitro* and promoted vascularization and osteogenesis *in vivo* with remarkably low doses of BMP-2 in a critical-size bone defect. The assembly of fibronectin on PEA has also been used to encourage vasculogenesis, by co-localization of integrin (α_v) and VEGFR-2 for synergistic integrin/VEGF receptor signaling (Fig. 8).[306] High stimulation of proteins activated by VEGFR-2 was only observed when the bound VEGF was presented in close proximity to integrin-binding sites. This agrees with the observation that phosphorylation of the VEGF receptor-2 was prolonged in association with integrins,[267] and that ERK1/2

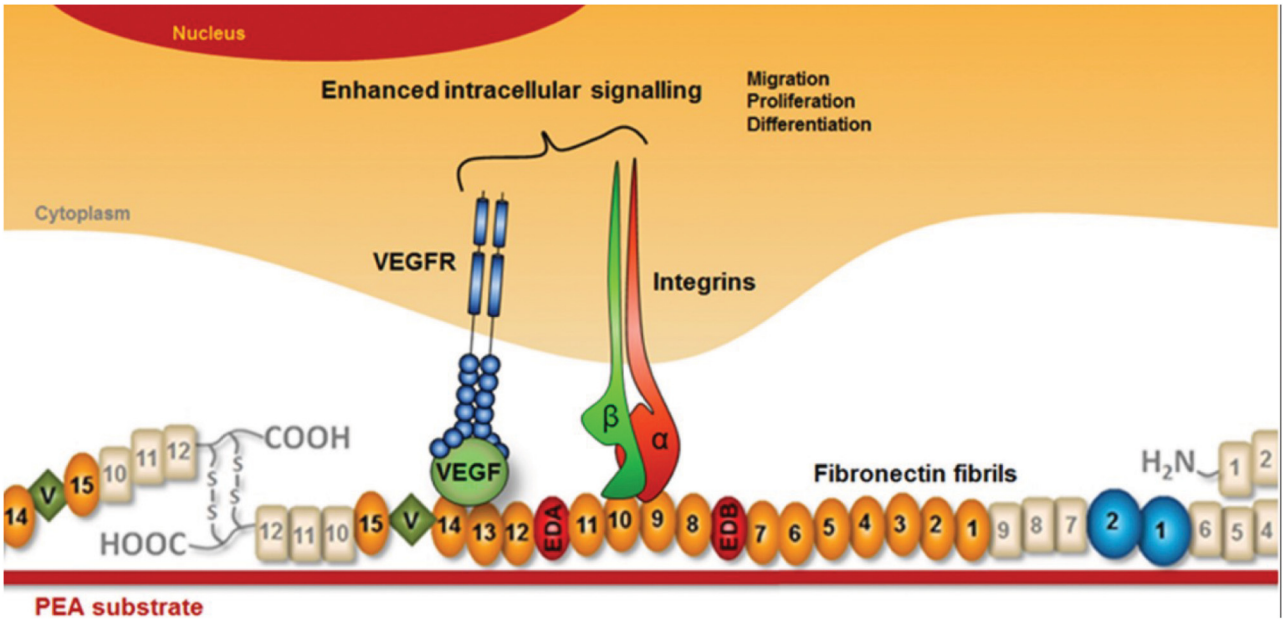

FIG. 8. Integrin-VEGF synergistic signaling triggered by FN organized into nanonetworks on PEA: the presentation of VEGF bound to FN in close proximity to the integrin-binding site effectively enhanced outside-in signaling by allowing VEGFR and integrins to work in synergy. Reprinted with permission from Moulisová *et al.*, Biomaterials **126**, 61 (2017). Copyright 2017, Elsevier.

(crucial for gene expression and DNA transcription) was phosphorylated as a result of activation of both integrins and VEGFR-2 based ERK cascades.[306]

Different surface immobilization techniques and controlled local delivery of GFs have also been investigated. For example, materials that incorporate GFs have been engineered to be protease sensitive, presenting a cleavable site between the growth factor and the heparin-binding site in order to induce release upon cellular demand.[307–309] Recently, Shekaran *et al.*[310] developed a poly(ethylene glycol) (PEG) hydrogel functionalized with a collagen-mimetic $\alpha_2\beta_1$ integrin-specific peptide (GFOGER) and cross-linked with MMP-degradable peptides to deliver BMP-2 in non-healing bone defects. These hydrogels provided continuous release of low doses of GFs *in vivo* and efficiently supported osteoprogenitor cell recruitment to the defect site due to effective MMP-dependent gel degradation.[310] Enhanced bone formation with defect bridging and mechanically robust healing was induced only in the presence of hydrogel implants incorporating GFOGER and BMP-2, even in low dose. A similar synthetic hydrogel, utilizing VEGF as the signaling molecule, was developed by García *et al.*[309] to enhance vascularization and bone regeneration. PEG hydrogels cross-linked with protease-degradable bi-cysteine peptides were functionalized with either $\alpha_2\beta_1$ integrin-specific peptide (GFOGER) or an $\alpha_v\beta_3$ integrin-targeting peptide (RGD). Covalent incorporation of VEGF into the PEG hydrogel allowed for protease degradation-dependent release of the GF while maintaining bioactivity. Increased dose of VEGF was followed by an increase in vascularization using both integrin ligands, but with levels of vascularization for VEGF-containing RGD hydrogels similar to those of VEGF-free GFOGER. However, addition of VEGF to these hydrogels did not enhance bone repair in a bone defect model. This study demonstrated the importance of integrin specificity in engineering biomaterials for vascularization and bone regeneration. It is likely that we will see materials presenting multiple types of GFs and or integrin ligands in the future, to better promote complete healing.

Significant work has been conducted to engineer materials that combine different matrix stiffness with GF presentation to better understand the interplay between mechanotransduction and GF-receptor signaling. Crouzier *et al.*[311] investigated how mouse myoblast C2C12 cells respond to BMP-2 presentation (soluble versus matrix-bound) using LbL-based surfaces with modulated stiffness. They found that cells responded to both soluble and matrix-bound BMP-2 on stiff surfaces, but only showed adhesive and migratory responses in the presence of matrix-bound BMP-2 on softer surfaces, remaining round and poorly spread in the presence of soluble BMP-2. It was proposed that when BMP-2 is bound to the film, active GF receptors are in the vicinity of adhesion receptors, enabling a cross talk between these two types of receptors. Thus, synergies between BMP-2 signaling and adhesion signaling might induce cytoskeleton remodeling and cell spreading. This cooperative response is not possible when BMP-2 is presented in solution. The same mechanism of stiffness modulated LbL films with matrix-bound BMP-2 was recently utilized to understand the biochemical interplay between integrin and BMP-2 signaling (Fig. 9).[1] In that study, it was found that matrix-bound BMP-2 was sufficient to induce C2C12 cell spreading in a β_3 integrin-dependent manner even on a soft biomaterial. $\alpha v\beta 3$ integrin was in turn shown to mediate Smad signaling upon BMP-2 stimulation through an Src–FAK– ILK axis, by inhibition of these cytoplasmic kinases. Additionally, it was demonstrated that β_3 integrin regulated Smad stability by negatively regulating GSK3 (glycogen synthase kinase 3) activity though the β_3 downstream effector, ILK. GSK3 inhibition is required for Smad phosphorylation and the Smad-associated transcriptional response, both of which are important for the osteogenic differentiation. The authors concluded that BMP-2 receptor and β_3 integrin signaling converged to control both focal adhesion dynamics and Smad pathway activation to coordinate cell migration and fate commitment.

The correlation between stiffness of the environment and BMP-2 has also been studied in mesenchymal stem cells.

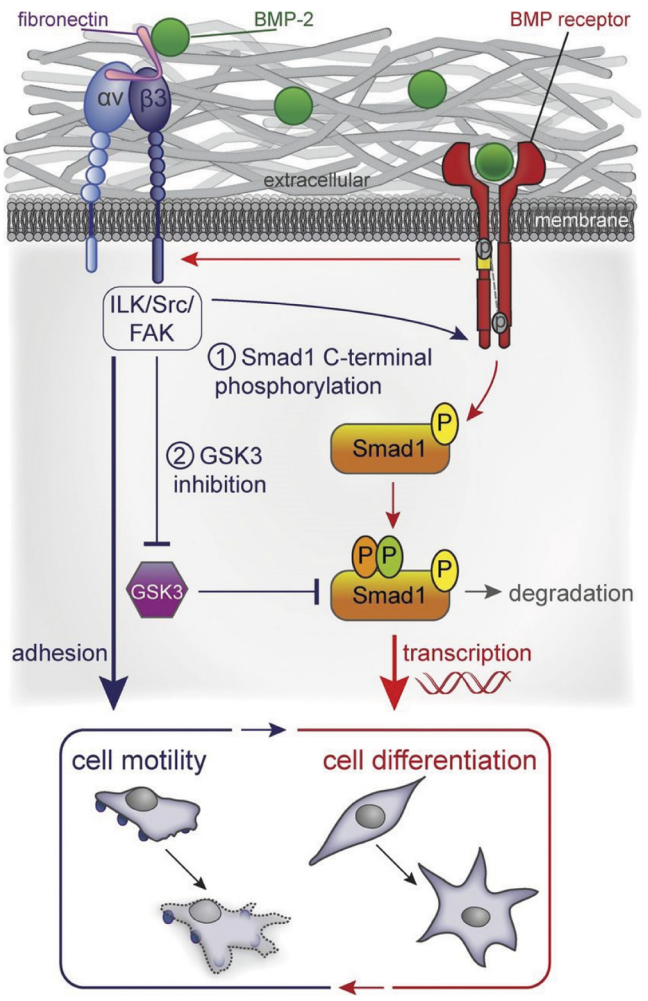

FIG. 9. Schematic view of β_3 integrin and BMP-2 signaling cross talk to control both focal adhesion dynamics and Smad signaling to couple cell migration and fate commitment. Reproduced figure from Fourel *et al.*, J. Cell Biol. **212**, 693 (2016). Copyright 2016, Rockefeller University Press.

Zouani *et al.*[312] used poly(acrylamide-co-acrylic acid) hydrogels with a stiffness ranging from 0.5 to 70 kPa grafted with adhesion ligands (RGD peptide) and a BMP-2 mimetic peptide to study the effect of mechanical and biomolecular factors on MSC differentiation. The substrate stiffness alone directed commitment of MSC to specific lineages, with myogenic differentiation occurring at 13–17 kPa and osteogenic differentiation at 45–49 kPa. When grafted with BMP-2, it was found that cells were committed to osteogenesis when cultured in gels with rigidity above a minimal threshold (≈3.5 kPa). The presence of GFs on very soft microenvironments (0.5–3.5 kPa) had no effect on MSC differentiation.[312] Note that BMP-2 was observed to inhibit the effect of the substrate stiffness for gels with intermediate stiffness (13–17 kPa). Similar results were found by Banks *et al.*[313] using matrices of polyacrylamide with photo-tunable elastic moduli of ∼5, ∼14, and ∼37 kPa combined with immobilized BMP-2 to determine adipose-derived mesenchymal stem cell lineage differentiation. It was observed that BMP-2 had the most pronounced effect at the intermediate stiffness, displaying the greatest up-regulation in expression of osteogenic specific marker genes, while the lowest and highest stiffness hydrogels were directed by elasticity alone. The highest stiffness substrates led to significantly elevated expression of bone-specific markers and a significant decrease in expression of fat-specific markers, consistent with the literature.[10,314] Little change was observed on the lowest stiffness substrates compared to the nonfunctionalized control. Both studies prove that above a certain substrate stiffness, MSCs are inclined toward an osteogenic phenotype, in the presence or absence of BMP-2. However, at intermediate material stiffness, the presence of a biochemical factor modifies the effect of the substrate stiffness on stem cell commitment.

The link between GF sensitivity and substrate mechanics has also been demonstrated in the context of dermal wound healing. Wickert *et al.*[315] showed that keratinocyte sensitivity to EGF was observed only on high stiffness PAA gels (>30 kPa), even though EGF receptors were found phosphorylated in cells on all substrates stiffnesses investigated, indicating that receptor activation is necessary, but not sufficient, for keratinocyte differentiation and proliferation. Since wounds have low stiffness immediately after injury (<20 kPa) with increase over time,[316] this study suggests that the use of EGF as therapy would be more efficient if applied at later stages of wound healing, emphasizing the importance of developing therapies that are context-specific and take into consideration the dynamics of biological processes.

Mechanisms that combine gradients of GF concentration and matrix stiffness are only starting to be developed. Garcia *et al.*[317] engineered a microfluidic device that enables the combination of a stable chemical gradient and an orthogonal linear stiffness gradient over a hydrogel. The device was validated with Madin Darby Canine Kidney (MDCK) cells, epithelial cells that grow in tight monolayers, but undergo consistent cell dispersion when in epithelial-to-mesenchymal transition induced by hepatocyte growth factor (HGF). It was observed that MDCK cell scattering was dependent on the synergy between HGF concentration and substrate stiffness. This engineered device promises to be particularly useful in high throughput assays in which the interplay between chemical and mechanical conditions needs to be tested.

To summarize Sec. VI, in reviewing the literature on model systems developed to study the synergies between mechanotransduction and GF signaling, it is clear that stiffness has been the prevailing cue to regulate mechanotransduction thus far, using gels or gellike systems. While other approaches to controlling mechanotransduction, such as ligand density, will be important contributors to the field, gels have the advantage that they offer a simple method to incorporate soluble GFs to be released. Gels are also available for cell experiments in 2D and 3D systems and mimic important elements of native ECMs.

VII. CONCLUSIONS AND PERSPECTIVES

In this review, we have discussed mechanotransduction and growth factor signaling *in vivo* as well as how engineered interfaces have been applied to learn more about these processes. It is important to have an understanding of how signals are transduced from the outside of the cell to the inside, and what kind of signaling cascades that follows. To transfer mechanical information across the floppy cell membrane, cells use assemblies of transmembrane integrin proteins, linked to the cytoskeleton via a raft of intracellular proteins. It is not only the mechanical force itself that is transduced, it is also turned into biochemical signals by a range of kinases, with focal adhesion kinase as a particularly central player. These initiated signaling cascades are central to maintain optimal cellular tension and rigidity sensing, and have also been shown to affect nuclear translocation of transcription factors. Taken together, a cell's response to the mechanical properties of their surrounding has implications for cell mobility, differentiation, tissue remodeling, homeostasis, and even cancer progression. Growth factor receptors are also located in the cell membrane, often close to the integrin clusters involved in mechanotransduction. Growth factors are soluble proteins central to cell-cell and ECM-cell communication. These potent proteins are often found bound to the ECM, and cells gain access to these while applying mechanical forces to and degrading their surrounding matrix. Thus, the link between mechanotransduction and growth factor signaling is clear, but the diversity of integrins, matrix ligands, GF receptors, and GFs means that cross talk and synergies between these two systems can occur at many levels. A better understanding of these interactions is needed, in particular to facilitate complete control over cell fate *in vitro*. By continuing to apply engineered model systems to answer fundamental biological questions and applying that knowledge, better control of cellular behavior *in vitro* is on the horizon. However, in an interdisciplinary field, care must be taken how results from simplified model systems are interpreted and generalized. It is encouraging that this field is becoming more and more cross-disciplinary, with collaborations and contributions from teams connecting medical scientists,

biologists, physicists, chemists, and engineers. Previous technological developments have provided interfaces with well-defined patterns or stiffness, which revolutionized how biological questions can be answered. To improve *in vitro* control over stem cell fate, research has studied effects of surface tethering of GFs to localize the GF close to the focal adhesions giving rise to synergy due to GF-receptor and integrin co-clustering. Increased efficiency has also been attributed to prolonged presentation. However, tethering of growth factors may change the downstream signaling by restricting receptor internalization, and the effects of GF internalization and receptor co-clustering remain challenging to separate. Therefore, as a scientific community, we need new, cleverly designed model systems able to differentiate the effects of localization of cell signaling molecules on cellular behavior from the effects of ligand tethering. Thus, engineered model systems are expected to continue to be central in providing key insights into cellular processes in general and stem cell regulation in particular. A significant challenge also exists in establishing if the understanding derived from model systems holds significance *in vivo*. For example, to design a growth factor delivery system, the main goal is to deliver sustained low doses of bioactive growth factors at a precise location. Delivery would ideally be achieved with complete spatiotemporal control—perhaps in response to particular biological events. With any GF eluting *in vivo* application, the aim is to decrease the effective dosage of GFs used, relevant to prevent off-target effects in clinical applications.

To fully exploit the promise of stem cells as therapeutic options, there is a need to ensure complete control over cell fate in all stages of the process. Ideally, such control should be ensured by delivering signals with spatiotemporal control to the cells from the synthetic material *in vitro*. Building on decades of elaborate surface engineering and characterization, the time is now ripe to develop systems allowing for precise and dynamic control over several properties simultaneously. There are exciting developments in taking time-dependent material properties into account,[242,318] creating intricately light-controlled activation of cell force,[319,320] creating dynamic organoid systems,[318] and achieving spatiotemporal control of hydrogels[321] to mention a few. Recent work demonstrating patterning and spatiotemporally controlled release of proteins from hydrogels is an excellent example of achieving control over several important cues in an orthogonal manner.[322] Material developments are of course enabled by advances in synthetic chemistry, for example, orthogonal coupling chemistries,[323,324] but are also importantly underpinned by characterization techniques being constantly improved. Developments within surface characterization, super-resolution microscopy, and functional imaging, such as advanced atomic force microscopy, are continuously being made and applied in the field of functional biointerfaces.[325]

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Jenny Malmström is a senior lecturer in the Department of Chemical and Materials Engineering at the University of Auckland. Her research focusses on creating functional biointerfaces to understand and control biological systems. She never expected to become an academic and did not know what a Ph.D. was growing up. In fact, she used to want to become a rubbish truck driver. She and her sister were the first in the family to attend University, so she had the pleasure of growing up with low expectations, but a great deal of support. Her passion for experimental science started early. The first exposure to a "real" laboratory was at the end of high school when she called up a professor at Stockholm University and asked to spend a week in their lab as an intern. There, she was introduced to growing nitrogen fixating bacteria and learnt what a Ph.D. student is.

When she started to study Bioengineering at Chalmers in Gothenburg, Sweden in 1998, it was the first time she experienced that learning was hard. After overcoming the significant struggle of the first year, she took a year off to work and travel before returning to university. She graduated with an M.Sc. of bioengineering in 2004 after traveling to New Zealand to perform her final research project. That project gave her a taste for independent research, leading into a one-year postgraduate research programe. During that programe, Jenny worked in three different research groups and got a good insight in what research she enjoyed and the importance of a good supervisor. One of the projects during that postgraduate year was with Dr. Duncan Sutherland, who subsequently secured a position at the University of Aarhus in Denmark and offered her a Ph.D. position, which she took up.

After her Ph.D., she decided to go to Auckland in New Zealand for a postdoctoral research position in polymer chemistry. To do this, she applied for all postdoctoral funding she was eligible for. While all of her applications were unsuccessful, her determination secured her a locally funded postdoc. Moving to a new field after her Ph.D. was both rewarding and challenging. It was a very valuable way to expand research skills. In 2016, Jenny was successful in securing a permanent academic position at the Department of Chemical and Materials Engineering. That year, she was also awarded two major early career research grants. Taken together, this was a significant event in her career, providing the security, funds, and research time needed to start her research group. She is currently supervising a talented group of Ph.D. students and honors students. The research in the group is all about interfaces: The interfaces where biological molecules meet novel materials and the interfaces between biology, chemistry, and materials science. The group's expertise in characterizing and understanding the material-biomolecule interactions is applied to emerging and exciting areas such as the creation of smart materials to help understand or control cellular behavior.

During Jenny's meandering path, a few pieces of advice have stood out as central:

(1) It is important to find a Ph.D. topic you are passionate about, but it is possibly even more important to choose your Ph.D. supervisor with care. My Ph.D. supervisor, Prof. Duncan Sutherland, was central in my career as he made me believe in my ability and shared his inspiration for the biointerface field with his students.
(2) Persistence and patience is key in research and in particular, when it comes to applying for grants and positions. Listen carefully to feedback and advice to improve, but do not stop applying!
(3) Some of the best advice I have had, from a female researcher, is that there is never a good time to have kids—so there is little point waiting for the perfect time. I never wanted to choose between having kids or a career, and had my first child during my Ph.D. and my second during my first postdoctoral position. For the young women out there who want both a family

and a career, it is possible and a supportive partner makes all the difference!

Isabela Monteiro commenced her doctoral studies at University of Auckland in November 2016, combining synthetic materials with biological molecules to better understand stem cell behavior. She has a Bachelor of Science and Technology with specialization in Biomedical Engineering from the Federal University of Sao Paulo, Brazil. After one and a half year of internships at large multinational companies, she realized that her major interest was to follow an academic career in science. In her Ph.D. research conducted with Dr. Jenny Malmström, Isabela applies advanced materials characterization techniques to investigate cell responses to micro- and nanoengineered surfaces.

Tarek Kollmetz is a Ph.D. student in Jenny Malmström's group at The University of Auckland, New Zealand. He received his M.Sc. degree in Nano Science at the University of Hamburg in Germany 2017. During his Master's, he conducted research on block copolymer physics at Helmholtz Centre Geesthacht in Germany. His current research focusses on the controlled release of proteins from polymer thin films for applications in cell studies.