Three-dimensional mapping of electrical propagation in the heart: experimental and mathematical model based analysis

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Abstract

In this thesis, mathematical modelling, instrumentation development and experimental studies are combined to effect two advances in the study of cardiac electrophysiology. Firstly, we outline a method that will enable microstructural information gathered using extended confocal microscopy to be incorporated into whole heart models of electrical propagation. A structured finite element technique is used to solve the bidomain equations on a realistic representation of myocardial architecture. The effects of intercellular gaps in the tissue on electrical propagation, and the response of myocardium to defibrillation-strength shocks, are examined. It is shown that myocardium is not a transversely isotropic electrical medium as has been widely assumed. Instead it is most appropriately viewed as orthotropic, with different electrical properties assigned to three microstructurally defined orthogonal axes. It is also concluded that the intercellular tissue gaps provide a likely mechanism for activation of a critical volume of tissue during defibrillation.

Secondly, a novel imaging system is presented which enables transmembrane potentials to be recorded simultaneously at multiple sites through the heart wall. While heart surface optical recordings of transmembrane potential have been widely used in studies of normal and pathological heart rhythms, it is difficult to obtain information about propagation processes deep within the heart wall. A probe constructed from optical fibers is used to deliver excitation light to and collect fluorescence from 6 tissue sites each spaced 1mm apart. For individual channels, excitation is provided by the 488nm line of a water-cooled argon-ion laser, while the fluorescence of a voltage-sensitive dye is split at 600nm and imaged into separate photodiodes for later signal ratioing. The system has been sucessfully used to record intramural action potentials in the isolated rabbit heart and is designed to be easily expandable to accommodate multiple optical probes. The fluorescence collection volume of the fibre-optic probe has been characterised in rhodamine solution using two-photon microscopy. These data are compared with corresponding results obtained with two flat cleaved optical fibres in solution and in stained heart tissue. The results demonstrate that the effective collection depth in cardiac

tissue for our optrodes was 100µm, but that this is dependent on the type of optical fibre used to fabricate the optrode.

These techniques developed in this research provide a basis for more systematic study of the initiation and termination of reentrant electrical activity in the heart. Future research that builds on the original findings presented in this thesis is discussed.

Preface

The research outlined in this thesis comprises two major bodies of original work: (i) development and analysis of a bidomain model of electrical propagation which incorporates accurate data about the microscopic architecture of the heart, (ii) development and characterisation of a novel experimental technique for measuring transmembrane potential at multiple sites through the heart wall. This work involved three seperate studies which form the basis of manuscripts that are currently published, submitted, or in preparation for peer reviewed scientific journals. These are:

D.A. Hooks, K.A. Tomlinson, S. Marsden, I.J. LeGrice, A.J. Pullan, B.H. Smaill, and P.J. Hunter. Cardiac microstructure: implications for electrical propagation and defibrillating the heart. *In preparation for Circulation Research.*

D.A. Hooks, I.J. LeGrice, J.D. Harvey, B.H. Smaill. 2001. Intramural multisite recording of transmembrane potential in the heart. *Biophys. J.* 81:2671-2680.

D.A. Hooks, D.A. Wardle, C. Soeller, I.J. LeGrice, B.H. Smaill, M. Cannell. Spatial point response function of fibre optic probes determined by two-photon microscopy. *In preparation for Applied Optics.*

These three manuscripts form the basis of chapters 2, 3 and 4, in this thesis. Additional explanation and background information has been added in these chapters where this was judged to be necessary. The work involved inputs from a number of people and this is acknowledged in the authorship of the manuscripts. Further detail of the valuable assistance which I have received is provided below. However, I was the principal investigator in each of the three studies, coordinating and directing most aspects of the work.

Chapter 1 provides an overview of the general field addressed in this thesis. *Chapter 2* presents the mathematical formulation of the bidomain equations used in a discontinuous structural model of electrical propagation in the heart. Results of the model in both propagated and shock induced cases are examined. The transmural volume image of the left ventricle was obtained by Young et al. (1988) and initial segmentation was done by Scott Marsden as part of a final year project in Engineering Science. Together with Dr. Karl Tomlinson, we developed a finite element technique that enabled structural discontinuities to be represented as no flux boundaries within the context of a bidomain formulation. Results of the model in both propagated and shock induced cases are examined. *Chapter 3* presents a novel method which utilises the voltage-sensitive dye di-4-ANEPPS, and a novel recording system and probe *(optrode)* to make simultaneous measurements of transmembrane potential through wall of the rabbit left ventricle. The optrode concept was developed by myself, and Dr. David Wardle of the Physics Department had input toward the overall design of the optical system. *Chapter 4* follows with characterisations of the depth of fluorescence collection of the optrode and other fibre based probes. Dr. Cristian Soeller gave instruction and advice on the two-photon microscope. Finally, future directions for all aspects of the work are presented in *Chapter 5*.

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I wish to thank in particular David Wardle for endless support and help toward the optical parts of the thesis. Your enthusiasm and wisdom was always much appreciated. The optical set-up and methods also benefited greatly from the wisdom and support of Christian Soeller, John Harvey, and Mark Cannell. Also, thanks to Karl Tomlinson, for his excellent assistance in the modelling part of the thesis, along with the great help of Scott Marsden in coding the model. From the beginning, I enjoyed the company of Socrates Dokos, who could always offer insights on just about any topic, and Gregory Sands who has kept a watchful eye on proceedings throughout. Thanks Adele for providing new inspiration to the lab, and Dean Tai for tiding us over with beautifully constructed optrodes.

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geeky things. A big thanks also to the UnEmap team – David Bullivant, Duane Malcolm, and Jim Williams - I'm sure I gave you a few late nights, and tormented you often enough with phone calls from the lab. You can now rest. Thanks to Rob Kirton for telling it like it is. I hope you shake the hell out of those trabs and can write up soon. Good luck also to Marie Ward – you can't be far off, and I promise I wont leave a mess around the weigh balance ever again. To Arthur Burgess, I know you are pleased I have finished … no need to cry ooohh noooo again. I am training Adele in the ways of nagging though – watch out.

Finally, to all those outside of work who have walked with me along this PhD path. Goddy, Albert, Sally, Karla, Brian – I have to say you have not really shown much interest in the content of the research, but have always been most concerned as to when it will finish. Well, it has. Yes Brian, I know I said I only had 2 weeks to go about this time last year.

Most of all, thank you to my parents to whom this thesis is dedicated. They have never faltered in their support for whatever I turn my hand to. Thanks Mum for hurrying me along at the end (sorry I couldn't have beaten you though), and thanks Dad for always understanding more than any other non-scientist!

Contents

List of Figures