

Cardiac Research at the Interface of Engineering and Computing

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The UK's long history of innovation and leadership includes the first legislation worldwide on animal handling: laws against cruelty to animals were passed as early as in the late 17th century! In 1876, the Cruelty to Animals Act, related to any interventions considered to cause pain to living animals, was introduced, followed in 1986 by the Animals (Scientific Procedures) Act that governs modern biomedical research in this country.

The three Rs

Principal aspects of appropriate experimental technique are often referred to as 'the three Rs':

- **R**eplacement of research on living animals by other experimental models systems (from cell to man),
- **R**eduction in animal usage by use of advanced experimental designs and techniques,
- **R**efinement of procedures to eliminate/minimise animal suffering or distress.

It is evident that good laboratory practice and adherence to the three Rs is a self-interest of biomedical research, as only well-designed and optimally performed studies will yield relevant data. Advanced engineering and computational techniques, in particular, make significant contributions to the life sciences in this context.

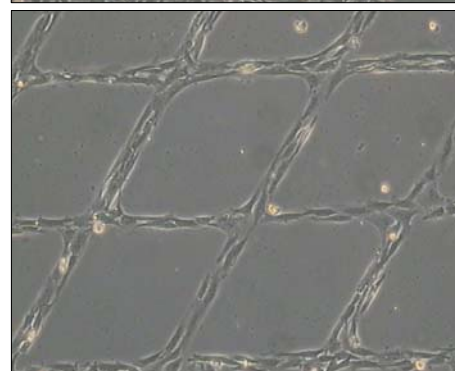
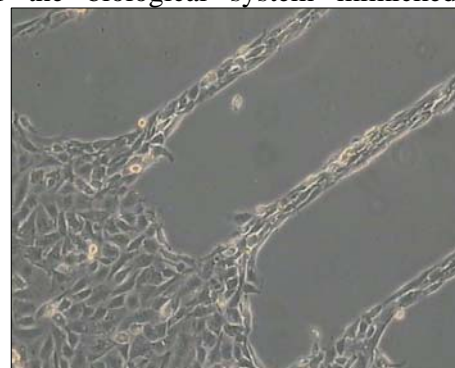
What are the demands for an ideal test-bed for drug testing, for example? Surely, it would have to be i) simple, ii) reproducible, and iii) relevant. *Simplicity* involves easy maintenance and low cost in terms of resources, specialist skills, and time requirements. *Reproducibility* entails standardised (ideally electronically portable) designs that can be reliably re-created with minimal training anywhere in the world. *Relevance* requires models that are representative of the biological system mimicked. Experimental models of the heart should represent cellular electro-mechanical characteristics, intercellular communication, and key features of cardiac structure.

The benefit of such a test-bed would be obvious in terms of scientific progress, resources, and both ethical and financial costs. The overwhelming majority of otherwise promising lead drug developments for any organ system is rejected, by the European and US regulatory bodies, for actual (or perceived) side effects on *cardiac* electrical function (an estimated loss of \$350m in R&D costs per drug rejected!).

The perfect model for cardiac research does not exist. Not yet.

Leading models, in terms of *simplicity* and *reproducibility*, are isolated cardiac cells. They are valuable experimental tools for the study of sub-cellular mechanisms and drug effects. But, they do not capture the spread of electrical activity that gives rise to both the normal heartbeat, and its disturbance. So, their *relevance* is limited to the lower levels of functional integration.

Cell cultures offer this interaction of thousands of cells. The commonly used mono-layer cultures, however, lack structural detail that would be representative of normal tissue. In these experimental models, cardiac cells quickly de-differentiate and lose many of their definitive features. Thus, the *relevance* of standard cell cultures is somewhat limited, too.



Cardiac cells grown on elastic membranes in user-defined patterns.

The flat heart

Fortunately, there is light at the end of the tunnel. Application of modern engineering techniques to cell growth and maintenance has improved cell culture properties in recent years. The ‘dull monolayer’ has turned into a 2D representation of cardiac structure and electro-mechanical function: a flat heart!

Researchers from Professor Andrew McCulloch’s lab at UCSD have applied micro-fluidic techniques to deposit patterns of extracellular matrix proteins on elastic membranes, to pre-determine cell attachment and growth in cardiac cell cultures. This provides more physiological growth conditions and significantly reduces de-differentiation of cardiac cells (see [3] for detail).

Our lab has been fortunate to be given early access to this exciting new technique, and we have extended the parallel lines of myocytes (essentially a 1D model of the heart) to criss-cross patterns of cardiac cells on elastic membranes. By varying the intersection angle of the parallel sets of lines, we can mimic differences in prevailing fibre orientation in our cardiac discs, which is a major determinant of the relative speed of electrical signal conduction and, hence, re-circulation of electrical waves.

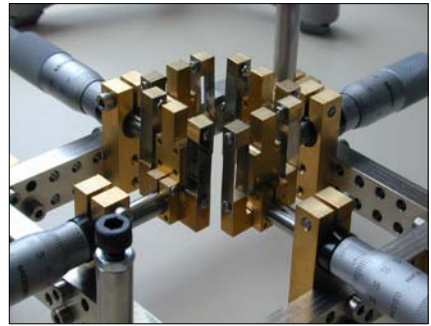
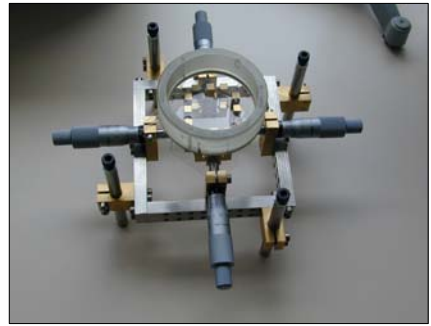
Given that these flat hearts are only a single cell deep, it is possible to optically monitor not only the mechanical activity of individual cells, but – using suitable bio-fluorescent dyes and high-gain photodiode systems – also any physiological parameter for which suitable dyes exist (such as electrical signals, ion concentrations, etc.). One can overlay this functional fluorescence signal with simultaneously obtained CCD images of underlying tissue structure in a fairly non-invasive way (a drawback remains in that fluorescent dyes interact with the measured entity, and thereby affect the very signal studied; a simplistic ‘Heisenberg’ for biologists...).

Nonetheless, this type of cell culture offers major advantages over the previous non-structured or static models of cardiac function. The micro-fluidic techniques applied allow seeding of cells in virtually any layout (provided it can be designed with a continuous flow-channel between an inflow and an outflow point), while the elastic membrane confers the ability to control the mechanical environment (note: all cells in the heart are normally subject to cyclically changing stresses and strains, and this affects their metabolism, growth, interaction and drug sensitivity).

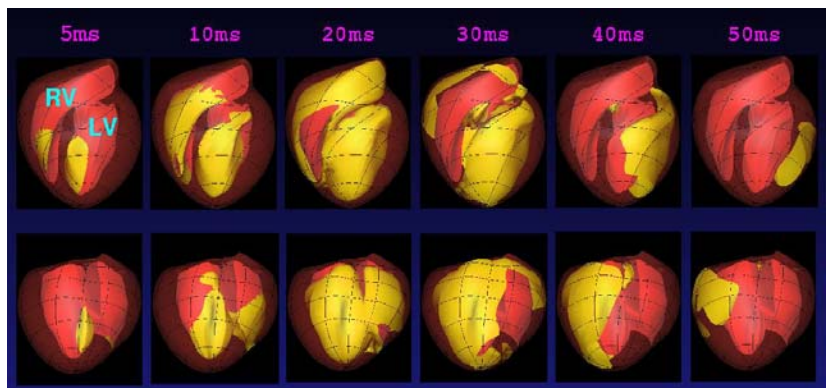
Thus, important aspects of clinical conditions, such as ischaemia or fibrosis, can in future be mimicked (by locally removing oxygen and metabolic substrates, or by super-seeding of connective tissue cells on otherwise normal myocyte tracks) in a *simple, reproducible* and *relevant* test-bed.

The virtual heart

An alternative to the above top-down engineering approach to ‘simplifying’ the heart is bottom-up computational integration of sub-cellular behaviour, obtained, for example, from isolated cell preparations. This overall middle-out strategy would potentially combine the best of both worlds: *simple* and *reproducible* experimental data sources, and physiologically *relevant* computational data integration. This principal idea has given rise to a major post-genomic Grand Challenge: the Physiome Project – an international effort to mathe-



Stretching apparatus for cell cultures grown on elastic membranes.



Electrical activation sequence in a 3D heart model (courtesy of P Hunter, Auckland).

matically describe biological function, from the molecular to the whole body levels (<http://www.physiome.org/>, see also [1] & [4]).

Computational modelling of the heart, in particular, has a long history, and UK scientists, notably Professor Denis Noble at Oxford, played pioneering roles in the development of single cell models for the heart and in the advancement of computational biology (<http://noble.physiol.ox.ac.uk/>, for representative papers see [5] & [6]). The most complex modern computer models of the heart combine realistic tissue architecture with detailed representation of cellular activity, coronary blood flow, and cardiac mechanics (exemplified by the work of Professor Peter Hunter's department at Auckland University, <http://www.bioeng.auckland.ac.nz/>). Suitably surface-rendered, these virtual hearts can even be made to look like a real beating heart, and one might be led to believe that 'the sky is the limit'...

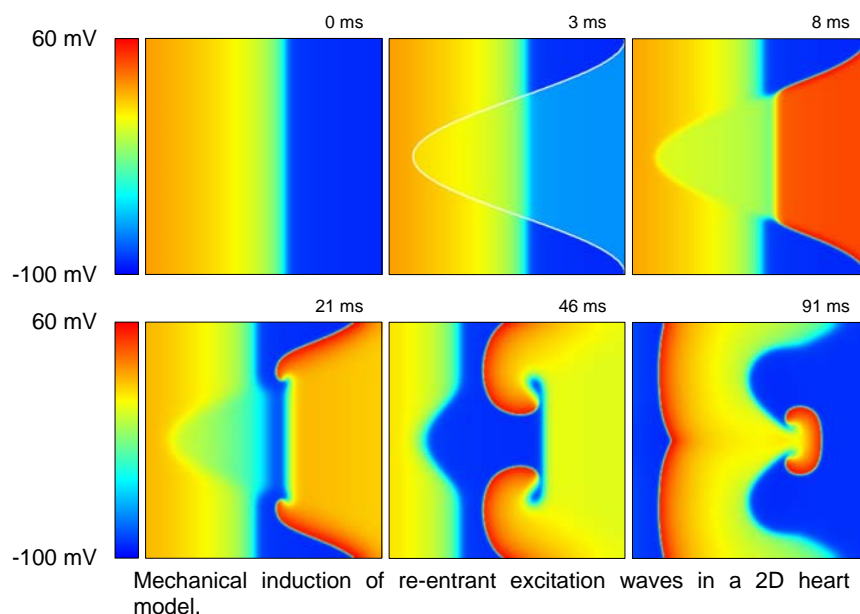
Indeed, the availability of ever faster computers could be seen as permissive for focussing on the development of the most complex models only. Indeed, the number of circuits on a computer microchip is understood to double *every 18 months* (Moore's law), so where is the problem?

The problem is that the amount of genomic data alone doubles *every 6 months*. The rise in the amount of 'reductionist' data, hence, continues to outstrip the increase in 'integrative' computing power by geometrically increasing orders of magnitude. A brute force approach to cardiac modelling is, thus, a lost battle right from the outset. As a rule, therefore, cardiac models should be made *as simple as possible, yet as complex as necessary* to address the actual question raised.

As in the case of 'wet' experimental studies, 2D cardiac modelling offers a valuable compromise for the theoretical investigation of many aspects of structure-function interrelation. An example is the recent identification of a potential mechanism underlying *Commotio cordis* – sudden cardiac death after low-force impact to the chest *without* apparent structural damage – that could explain the severe outcome. This condition can occur in athletes during sports such as baseball or ice hockey, when a baseball or puck hits a player in the chest. If this impact occurs just at the 'right' time (the vulnerable window lasts several milliseconds during each cardiac cycle), it may cause instantaneous death. This is a rare event, (some ten to fifteen occurrences per year in the US, for example), and underlying mechanisms are not well understood.

Previous research at the cellular level had already identified a number of important electrical responses to mechanical stimulation in single cells, but a clear understanding of their dynamic interaction was missing. We implemented a 2D model of a mechanical impact to the heart, which allowed us to identify a probable mechanism underlying both the devastating outcome of ill-timed mechanical stimulation and the shortness of the period of time during which *Commotio cordis* may cause sudden death. In short, not the induction of an extra beat, previously assumed to hold the key to the devastating outcome, but a rather 'innocent' local delay of impulse propagation is likely to lead to the development of re-entrant waves of electrical activity which, in man, would have the potential to cause sudden cardiac death (see [2]).

This somewhat counter-intuitive modelling-derived hypothesis is now the subject of experimental assessment (in structured 2D cell cultures), and serves to illustrate that the continued iteration between 'wet' and 'dry' studies provides a significant driving factor for progress in bio-medical research.



Mechanical induction of re-entrant excitation waves in a 2D heart model.

Outlook

Developments at the interface of engineering and computation hold tremendous promise for life sciences. This applies equally to fundamental and applied research. Advanced experimental and theoretical tools allow better study-design for more targeted research, thereby equally improving the cost-benefit balance and the *relevance* of our work. Novel *simplified* models enhance *reproducibility*, and form an invaluable addition to the more traditional ones, which in combination with international research strategies, allows for fewer and better studies involving responsible animal experimentation.

It is a requirement for international collaboration that experimental and computational models can be easily exchanged. This is greatly aided by the electronic distribution of related information. Computer modelling is now witnessing the development of an ‘Esperanto’ for bio-mathematics – new markup languages (such as CellML, <http://www.cellml.org/>) that allow platform-independent exchange of biological models and easy conversion between computing languages. As an example, the 2D model presented here was implemented using a new-generation environment (COR, <http://cor.physiol.ox.ac.uk/>) that is CellML capable.

Will mathematical models and simplified experimental tools make bio-medical research at the systems level redundant? The answer is a clear NO. Any prediction – whether the result of intuition, guesswork, quantitative assessment or low-level component testing – needs to be verified at the target level of integration. This can be illustrated by an important lesson from the Genome Project, where early hopes of solving malady via deciphering the human genome have not materialised and, instead, have given rise to such formidable post-genomic challenges as the Proteome and Physiome Projects.

Further reading

- [1] Crampin EJ, Halstead M, Hunter P, Nielsen P, Noble D, Smith N & Tawhai M. Computational physiology and the physiome project. *Experimental Physiology* **89**: 1-26 (2004).
- [2] Garny A & Kohl P. Mechanical induction of arrhythmias during ventricular repolarisation: modelling cellular mechanisms and their interaction in 2D. *Annals of the New York Academy of Sciences* (2004, in press).
- [3] Gopalan SM, Flaim C, Bhatia SN, Hoshijima M, Knoell R, Chien KR, Omens JH & McCulloch AD. Anisotropic stretch-induced hypertrophy in neonatal ventricular myocytes micropatterned on deformable elastomers. *Biotechnology and Bioengineering* **81**: 578-87 (2003).
- [4] Kohl P, Noble D, Winslow RL & Hunter P. Integrative modelling of biological systems: tools and visions. *Philosophical Transactions of the Royal Society, London A* **358**: 579-610 (2000).
- [5] Noble D. Cardiac action and pacemaker potentials based on the Hodgkin-Huxley equations. *Nature* **188**: 495-497 (1960).
- [6] Noble D. Modeling the heart--from genes to cells to the whole organ. *Science* **295**: 1678-82 (2002).

Authors

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