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SPOTLIGHT REVIEW

Combining wet and dry research: experience with model development for cardiac mechano-electric structure-function studies

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Since the development of the first mathematical cardiac cell model 50 years ago, computational modelling has become an increasingly powerful tool for the analysis of data and for the integration of information related to complex cardiac behaviour. Current models build on decades of iteration between experiment and theory, representing a collective understanding of cardiac function. All models, whether computational, experimental, or conceptual, are simplified representations of reality and, like tools in a toolbox, suitable for specific applications. Their range of applicability can be explored (and expanded) by iterative combination of 'wet' and 'dry' investigation, where experimental or clinical data are used to first build and then validate computational models (allowing integration of previous findings, quantitative assessment of conceptual models, and projection across relevant spatial and temporal scales), while computational simulations are utilized for plausibility assessment, hypotheses-generation, and prediction (thereby defining further experimental research targets). When implemented effectively, this combined wet/dry research approach can support the development of a more complete and cohesive understanding of integrated biological function. This review illustrates the utility of such an approach, based on recent examples of multi-scale studies of cardiac structure and mechano-electric function.

Keywords Heart • Mechano-Electric Feedback • Computational Model • Experimental Model • Multi-Scale

1. Introduction

The first biophysically based computational cardiac cell model was developed just over 50 years ago by Denis Noble.[1] Building on the pioneering work of Alan Hogkin and Andrew Huxley,[2] he reproduced the shape of an action potential (AP; change in transmembrane potential over time during cell activation) of a Purkinje cell by integrating mathematical formulations of three trans-sarcolemmal ion fluxes. Since then, cardiac models have moved on significantly,[3] and they are now among the best-developed theoretical representations of any organ,[4] distilling years of iteration between experiment and theory.[5] They benefitted from the fact that cardiac structure and function display a relatively high degree of spatio-temporal regularity, over multiple scales of integration (e.g. cell microstructure and transmembrane ion dynamics, whole-organ tissue organization, and patterns of electro-mechanical activation, etc.), and from a wealth of high-quality data (covering $>10^9$ length scales, from molecular to whole-organ levels, and a 10^{16} range in time, from nano-seconds to years). Cellular models included in highly detailed histo-anatomical representations of the whole-heart for *in silico* investigations of (patho-)physiological function[6] are on the verge of linking protein, cell, tissue, and organ behaviour,[7] to allow projection from genotype to phenotype.[8] Thanks to their advanced state, cardiac models are beginning to offer predictive (and thus clinically relevant) power.[9] This is helped by tools facilitating model implementation, such as resources for standardization and distribution,[10] as well as software for performing single-cell[11] and multi-cellular simulations.[12]

Thus, computational modelling is an increasingly productive tool for the integration of information on complex cardiac behaviour.[13] Any model, whether computational, experimental, or conceptual, is a simplified representation of reality. This very nature makes models useful for understanding complex systems.[14] Computational models must be validated against experimental data, and subsequently used in a context for which they are applicable.[15] This process benefits from an iterative combination of 'wet' and 'dry' investigation, as illustrated in *Figure 1*, where:

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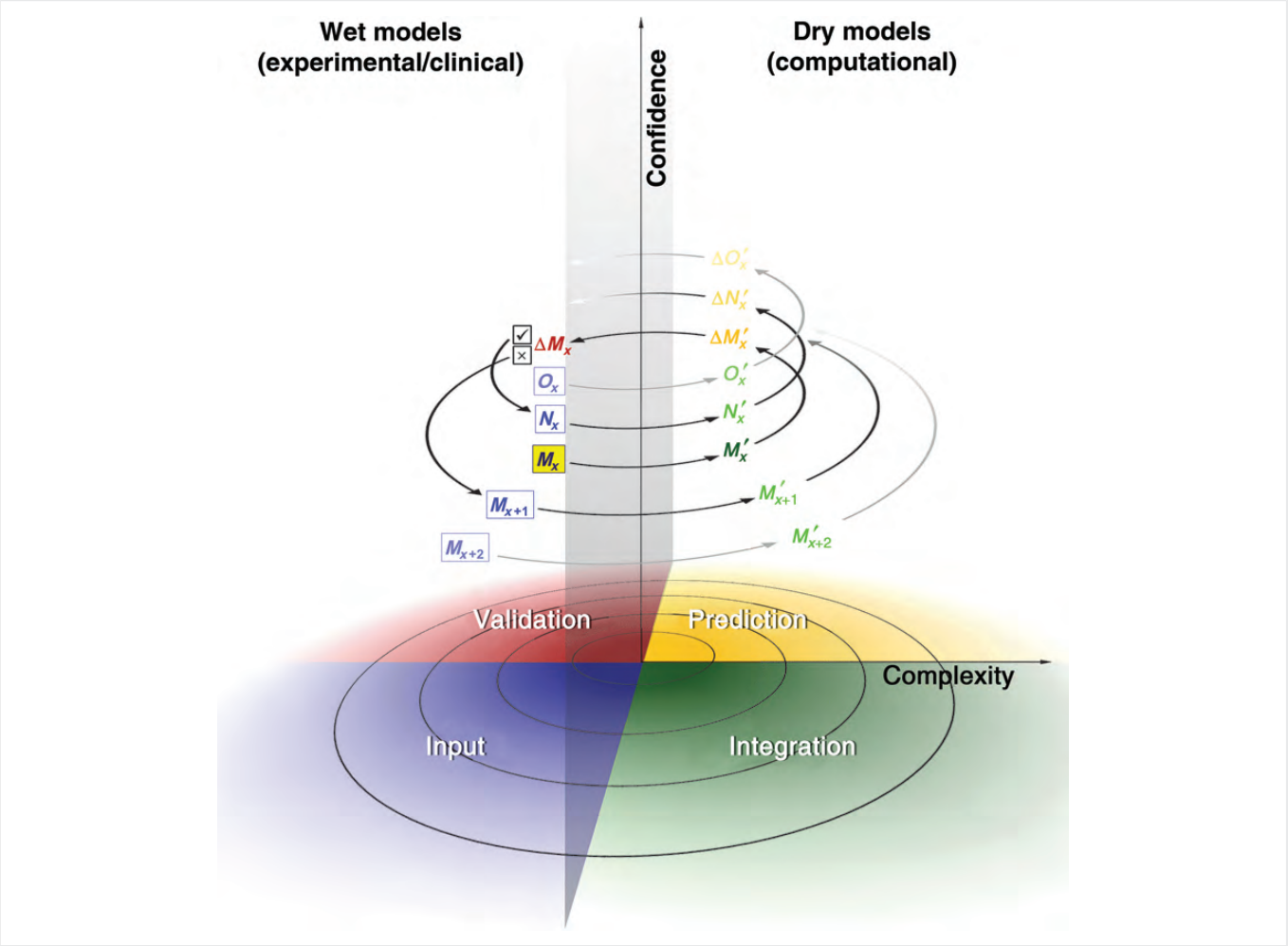

Figure 1 The iterative spiral of combined 'wet' and 'dry' research. Experimental and/or clinical data (M_x, highlighted in yellow, here serving as an arbitrary starting point representative of 'present-day insight') are used as inputs to build computational models (M'_x) for integration and interpretation of previous findings, and projection across relevant spatial and temporal scales. Simulations are then run with these models to generate novel predictions and hypotheses ($\Delta M'_x$). These undergo testing and validation, usually involving newly obtained experimental and/or clinical data (ΔM_x). When a mis-match exists between model predictions and new findings (☒), models are modified based on the newly obtained data (M_{x+1}), which may increase model complexity, and subjected to a repeat of the prediction/validation cycle. If instead there is agreement (☑), models can be used subsequently with increased confidence for the integration and interpretation of data (N_x). This iterative process proceeds continually, moving towards model improvement, and necessarily including a range of implementations with varying combinations of complexity (radial direction) and confidence (along the ordinate).

(i) experimental and/or clinical observations are used to build theoretical models, which are aimed at integration and interpretation of previous findings, and projection across relevant spatial and temporal scales (for integration from part to system to explore 'emergent properties', application of system-imposed boundary conditions to restrict constituent performance, and recognition of mutual causality between interacting mechanisms);
(ii) computational simulations are subsequently implemented to generate novel hypotheses and predictions, informing follow-up experimental design;
(iii) next, experimental and/or clinical studies are needed to validate (endorse or reject) model-derived hypotheses; and
(iv) newly generated insight is employed to further advance computational models.

Where experimental/clinical validation confirms computational predictions, subsequent modelling can be performed with increased levels of confidence. Where validation rejects model predictions, something *new* is learned, and further development and/or refinement must occur, improving models until validated. When implemented effectively, this iterative spiral allows a continuous move towards a more complete and cohesive understanding of integrated cardiac function.[16] Of note, this process may involve transition via more complex model representations than necessary, and subsequent model reduction to a level that is 'as simple as possible, yet as complex as necessary'[17] is a vital aspect to optimize the levels of confidence attributable to model predictions (*Figure 1*).

The purpose of this review is to illustrate the utility of combining wet/dry approaches as an integral part of the research process.

Examples are based on our experience with multi-scale, multi-modal investigations of cardiac structure and mechano-electric function.

2. Wet/dry integration: from micro to macro

Sub-cellular cardiac processes are integrated at the cellular level to produce electrophysiological and mechanical outputs that can be evaluated experimentally and computationally in single isolated cells. By altering the Hodgkin-Huxley formulation for neuronal cells to allow for trans-membrane potassium (K^+) flow through *two* types of channels (one in which conductance falls with membrane depolarization and another in which it slowly rises), Denis Noble was able in 1960 to reproduce the AP morphology of Purkinje cells.[1] The model incorporated only one voltage-gated inward current (conducting sodium, Na^+, as the inward calcium, Ca^{2+}, current had not yet been discovered). To reproduce an AP plateau, the model was 'tweaked' (a common practice in model development),[18] by increasing the voltage range of Na^+ current activation (so that, in essence, it accounted both for Na^+ and Ca^{2+} channel contributions to AP shape).

This need for model adjustment to reproduce experimental observations (essentially 'a failure' of the initial model) was highly productive, as it generated two novel hypotheses: either Na^+ channels in the heart had to be qualitatively different from those in nerve, or other inward currents had to exist. Soon after, both hypotheses were confirmed experimentally. Based on the application of the then new voltage-clamp technique to cardiac cells,[19] this contributed to the discovery of the L-type Ca^{2+} current by Harald Reuter in 1967.[20] So, while the Noble-1960 cardiac cell model was an (over-)simplified representation of actual physiology, it proved to be a useful tool for hypothesis-formation, driving further research. Since that time, biophysically detailed cell models have developed into more complete representations of sub-cellular processes,[21] being among the most detailed and well-tested models in integrative systems biology.[22]

Investigation of many important (patho-)physiologically relevant aspects of cardiac electrical and mechanical function requires multi-cellular and tissue-level considerations, such as related to connected cell electrophysiology, histo-anatomical structures, and spatio-temporal effects. Cardiac function involves the interaction of heterogeneous cell populations (e.g. cardiomyocytes, fibroblasts, neurones, pacemaker and endothelial cells) and other cellular and acellular components (e.g. blood, lymph, extracellular matrix). Native tissue preparations represent a model in which individual cells are in histo-anatomical conditions close to the *in situ* setting,[23] but where targeted intervention and observation at the cell-level are more difficult. The projection between cellular and integrated tissue behaviour can be significantly enhanced by pairing experiments with computational studies. Computational modelling of this setting (multiple cell populations with heterogeneous biophysical and biochemical coupling) is challenging,[24] and still based on a large degree of simplification.[25] In the ideal case, both experimental and computational models will be as simple as possible, to facilitate implementation and interpretation, but as complex as necessary, to ensure inclusion of all relevant factors, and finding a suitable balance remains perhaps the major challenge in this area to date.[17] The utility and implementation of a combined wet/dry approach for understanding and predicting experimental and clinical cardiac mechano-electric responses at multiple levels of spatial integration is illustrated in what follows.

2.1 Understanding physiology: effects of stretch on pacemaker function

It has long been known that mechanical stimulation of myocardium affects its electrical activity.[26] This has been studied in humans and whole-animals, isolated heart and tissue, single cells, and membrane patches. It is now understood that this is due to feed-back from the mechanical environment to the origin and spread of excitation (termed mechano-electric feedback, or coupling, MEC).[27] At the molecular level, MEC involves activation of specialized mechano-sensitive ion channels in sarcolemmal and intracellular membrane compartments, direct and secondary effects of stretch on the activity of ligand- or voltage-gated ion channels and transporters, and modulation of Ca^{2+} handling (such as secondary to mechanically induced changes in intracellular Ca^{2+}-buffering capacity of heart muscle).[27] Expressions of MEC have been reported for all cardiac cell types probed, including ventricular[28] and atrial[29] myocytes, fibroblasts,[30] and endothelial,[31] pulmonary vein,[32] or pacemaker[33,34] cells.

Understanding stretch-effects on cardiac pacemaker electrophysiology is of particular interest, as these cells play essential roles in beat-by-beat adaption of heart rate to changes in haemodynamic load.[35] From classic whole-animal studies it is known that distension of the right atrium (by increasing venous return) raises heart rate.[36] This increase is insensitive to denervation,[37] ablation of intracardiac neurons,[38] or adrenergic/cholinergic block,[37] demonstrating that intra-cardiac mechanisms that are independent of, and additional to, humoural and neuronal signalling are involved.

In isolated sinoatrial node (SAN) tissue, microelectrode recordings have shown that the positive chronotropic response to stretch is associated with a reduction in absolute values of maximum diastolic and maximum systolic potentials (MDP and MSP, respectively).[39] This could be explained by activation of a trans-sarcolemmal current with a reversal potential (E_{rev}) somewhere between MDP and MSP of SAN cells.

Early investigations into the mechanisms underlying mechanical modulation of pacemaker function utilized positive-pressure cell swelling as a mechanical stimulus. In rabbit SAN cells, this was shown to activate a chloride current (E_{rev} ~0 mV).[40] In theory, this could account for the changes in SAN electrophysiology and beating rate (BR), observed at the tissue level. However, studies using hypo-osmotic swelling of spontaneously beating cells showed a reduction, rather than the anticipated increase, in rabbit SAN cell BR.[41] This raised the question: what causes the unexpected change in BR during cell-swelling?

To avoid a 'fishing expedition', computational modelling was used to identify 'plausible' explanations. Simulating hypo-osmotic swelling in a biophysically detailed computational model of SAN cell electrophysiology,[41] it emerged that the observed reduction in BR could be driven by cytosol dilution. This, by decreasing intracellular K^+ concentration, reduced the delayed rectifier K^+ current (I_K), causing depolarization. In the model, this reduced the hyperpolarization-activated depolarizing ('funny') pacemaker current (I_f), lowering one of the driving forces for diastolic depolarization, and decreasing BR. This modelling-derived hypothesis was tested experimentally in voltage-clamped SAN cells, which confirmed the swelling-induced shift in E_{rev} of I_K, and a reduction in the amplitude of its rapidly activating component

(I_{Kr}).[41] This example demonstrates the utility of targeted computational exploration for interpreting unanticipated results, and the value of direct iteration between wet and dry model systems.

The different response of BR to cell stretch vs. swelling furthermore showed that the two stimuli are not interchangeable: they differ in micro-mechanical deformation characteristics (increased cell length and reduced diameter vs. negligible change in length, or even shortening, and increased diameter, respectively), lag-times (near-instantaneous response to stretch vs. tens of seconds delay between swelling and electrophysiological-effects), and pathophysiological context (beat-by-beat activity vs. altered osmotic pressure such as during/after ischaemia). Therefore, axial stretch was applied to spontaneously beating SAN cells, using a modified version[42] of the carbon fibre technique.[43] This caused a significant increase in BR, accompanied by a reduction in the absolute values of MDP and MSP.[33] Subsequent voltage-clamp studies revealed stretch-activation of a whole-cell current (E_{rev} ~-11 mV), suggesting that cation non-selective stretch-activated channels (SAC_{NS}) may underlie the response.[44,45] These rapidly activating channels depolarize cells in diastole, while repolarizing them in systole. Their involvement was corroborated by reversible block of stretch-induced BR changes in guinea pig and murine SAN tissue, exposed to GsMTx-4 (*Grammostola spatulata* mechanotoxin-4, the most specific blocker of SAC_{NS} known to date).[46] Thus, computational modelling cannot only be used to evaluate conceptual models and guide experimental approaches, but also serve as a driver of new wet-lab tool development.

At the tissue level, understanding the effects of stretch on pacemaker function becomes more complicated. Other cells, including fibroblasts, may influence changes in BR,[47] as long as they express SAC_{NS},[48] alter their membrane potential with stretch,[30] and are electrically coupled to SAN myocytes.[49] It is necessary also to consider effects of heterogeneous tissue structure, electrophysiology, and mechanics, given that regional differences affect the extent of, and response to, stretch experienced by individual cells in different parts of the pacemaker tissue[50–52] (parameters that furthermore are age-,[39] species-,[53] and probably disease-dependent). Future computational and experimental research in this area will need to focus on an integrative approach to account for these tissue-level considerations.[35]

2.2 Explaining pathophysiology: mechanisms of mechanically induced re-entry

Changes in the mechanical environment also affect ventricular electrical activity.[54–56] Perturbations of the heart's mechanical status can occur as a consequence of intrinsic and extrinsic stimuli. Intrinsic stimuli include, among others, alterations in preload (discussed above in the context of pacemaker activity), and in intra-cardiac stress–strain distribution (discussed below in the context of whole-heart mechano-electric function). Extrinsic stimuli can be associated with invasive medical interventions (such as cardiac catheterization), or extra-corporeal impacts that may cause (e.g. *Commotio cordis*) or terminate (e.g. precordial thump) arrhythmias.

The electrophysiological effects of diastolic mechanical tissue stimulation (i.e. in tissue with cells at resting membrane potentials) are generally benign, causing cellular depolarization and, if supra-threshold, ectopic excitation.[57] As in pacemaker tissue, this can be explained by SAC_{NS} activation,[58] although this does not exclude other contributory mechanisms. Effects of systolic mechanical stimulation (i.e. during the AP) are more varied.[59] Stimulation during the AP plateau has a repolarizing effect, generally resulting in AP shortening. However, with continued cellular repolarization, the membrane potential eventually becomes negative to the E_{rev} of SAC_{NS}, such that maintained mechanical stimulation may give rise to late AP prolongation and cross-over of the repolarization curve, potentially leading to early after-depolarization-like events.[60]

The phase-dependence of stretch-effects on electrophysiology is an important factor in tissue, where trans-membrane potential distribution shows significant spatial heterogeneity during cardiac electrical activation and repolarization. This means that a mechanical stimulus, applied during the ECG QRS complex, but even more so during the T-wave, will not affect cells uniformly, as 'local AP' values will differ regionally across the heart. As the ventricular activation wavefront is steep and fast, it condenses regional heterogeneities into a very narrow temporal domain, and responses to mechanical stimulation (in particular if that stimulation gives rise to depolarization of excitable tissue) are therefore hard to detect. Ventricular repolarization, however, is slower and more graded. If the myocardium is mechanically stimulated during the T-wave, pronounced spatial heterogeneities in membrane potentials, refractoriness, and excitability may combine to promote arrhythmogenesis. This is particularly true if a mechanical stimulus affects, both, cells that have regained excitability (these may depolarize, giving rise to ectopic foci, thus providing a trigger for arrhythmogenesis), and cells at more positive membrane potentials (whose repolarization time-course is altered, potentially furnishing an arrhythmia-sustaining substrate).[61] While conceptually similar to the vulnerable window for electrical stimulation,[62] there is a *principally relevant difference* in that mechanical stimuli affect cardiac tissue in a localized fashion. This has implications for the pathogenesis of sudden cardiac death from *Commotio cordis* (mechanical induction of heart rhythm disturbances of varying severity, in the absence of structural damage to the heart that would explain observed functional changes).[63]

The vulnerable window for mechanical induction of ventricular fibrillation (VF) has been experimentally identified by Link *et al.*[64] in an anaesthetized pig model of *Commotio cordis* to be 15–30 ms prior to the peak of the ECG T-wave. Computational modelling offers a plausible explanation for this pronounced timing-dependence. Using simple two- and three-dimensional (2D/3D) models of ventricular tissue, with mechanical stimulation modelled via activation of SAC_{NS}, Garny *et al.*[65] showed that sustained re-entry was observed only if the stimulus: (i) encountered excitable tissue (giving rise to an ectopic focus by cellular depolarization), (ii) overlapped with the repolarization wave-end (giving rise to an area of functional block by local AP prolongation), and (iii) extended into tissue with membrane potentials above the E_{rev} of SAC_{NS} (giving rise to an arrhythmia sustaining substrate by regional AP shortening). Mechanical stimulation too early or too late for such overlap to occur was either inefficient in triggering excitation, or resulted in a single ectopic beat with no sustained, repetitive activation. Similar results have been shown using an anatomically detailed model of rabbit ventricles.[66] These computational predictions are now being tested by controlled application of non-traumatic local epicardial stimuli in isolated Langendorff-perfused rabbit hearts, while monitoring transmembrane potential distribution using fluorescent optical mapping.[67] It has been shown that sufficiently large local mechanical stimulation in diastole resulted in electrical activation from the site of mechanical contact (compatible with a local depolarizing effect of SAC_{NS}). Upon reduction of the delay for mechanical stimulation, relative to the preceding cardiac

cycle, ectopy-induction continued until stimulation coincided with the stage of repolarization when mechanically affected tissue overlapped the repolarization wave. This was the only setting in which induction of re-entry and VF could be observed, supporting the computational prediction and taking the iterative loop of wet/dry investigations to the next level.[68]

2.3 Predicting therapeutic outcomes: electrophysiological effects of ion channel block

Electrical and mechanical activity of the heart (and their mutual interactions) form key targets for pharmacological intervention, but even more frequently, they represent unwanted foci of drug side-effects. Among the main concerns for safety pharmacology are, therefore, unwanted cardiac responses to drug application. Among these, the possible induction of a ventricular arrhythmia known as *Torsade de Pointes* (TdP) represents a particularly challenging target. Occurrence of TdP is very rare (once in 10 000 patient-years!).[69] This, combined with frequently lethal outcome, makes prediction of 'torsadogenicity' of a drug both difficult and important. As a mandatory component of regulatory drug approval, tests for TdP-risk are a key part of pharmacological development, with expensive consequences: true- and false-positive results mean that time and money spent on drug development have been wasted (the average cost of bringing a new agent to market is estimated to be close to one billion USD),[70] while false-negatives have significant costs associated with patient risk and damage to company reputation.

To address this problem, pharmaceutical companies and regulatory bodies have devised rigorous (and expensive) drug-development and safety-profiling strategies that span from high-throughput ion channel screening, to *in vitro* cell, tissue, and organ assays, eventually reaching *in vivo* whole-animal studies, before drugs are taken to patient-testing. Their success hinges on biomarkers for identifying arrhythmogenic risk as early in the compound development process as possible.[71] Currently, one of the accepted early-stage biomarkers is the extent to which a drug blocks the human Ether-a-go-go Related Gene (hERG) channel.[72] The hERG channel is responsible for carrying I_{Kr}, whose block results in AP-prolongation and reduction of repolarization reserve, both potentially favouring arrhythmia-induction.[73] This motivated Redfern *et al.*[74] to correlate the degree of hERG-block with human clinical TdP risk, establishing the need for a safety margin equivalent to a 30-fold difference between effective therapeutic plasma concentrations of a drug and the concentration that causes 50% hERG-block (IC_{50}). However, correlation is not causation, and numerous currently used non-torsadogenic drugs would have been classified as carrying unacceptable TdP risks under the Redfern scheme (a false-positive that would have prevented safe drugs from reaching market). Vice versa, other drugs that have been shown to be torsadogenic would have passed the early hERG test (a false-negative that would result in their continued development).[69] This demonstrates the need for novel pharmacological screening methods to *predict* drug effects on cardiac electrophysiology.

While biophysically detailed computational modelling and simulation have been used for some time in drug discovery and design,[75–77] including the explanation of pharmaceutical effects on cardiac cell electrophysiology[78,79] and to understand therapeutic effects in diseases such as arrhythmia, ischaemia, and heart failure,[75] reliable prediction of arrhythmogenic risk, based on a minimum set of input parameters, remains challenging. That said, computational modelling is beginning to gain acceptance from the pharmaceutical industry and regulatory bodies as a potentially useful tool for improving the efficiency, accuracy, and confidence of pre-screening via rapid *in silico* testing of novel compounds, before more expensive wet-lab testing begins.[80]

The simplest way to simulate drug-induced ion channel block in computational formulations is by reducing the maximum value of the affected conductance (in most cases drugs affect ion channels by direct binding, obstructing ion flow either by forming a physical obstacle, by changing the conformation of the ion channel, or by affecting the probability of reaching an open state).[81] This is typically accomplished with a scaling factor that follows the dose–response curve of the drug (the relationship between drug concentration and degree of current reduction).[80] By incorporating multi-channel effects in whole-cell models, it is possible to explore drug-effects on AP configuration.[82] This shows that a compound that simultaneously blocks depolarizing currents (such as inward Na^+ and Ca^{2+} currents) and repolarizing currents (such as outward K^+ currents) to a similar extent may have little overall effect on AP duration. One such substance is ranolazine, an anti-angina compound that blocks both hERG and the late Na^+ current, resulting in a significantly smaller AP prolongation (and arrhythmic risk) than would have been expected from considering its effects on hERG alone.[83]

Computational modelling of whole-cell, multi-conductance block is showing good promise for pharmaceutical safety testing.[84,85] Thus, Mirams *et al.*[86] included experimentally established effects of drugs on three ion channels (hERG, fast Na^+, and L-type Ca^{2+}) into a human ventricular electrophysiology cell model. Using the model, prolongation of simulated APs for 31 currently marketed compounds was correlated with their known clinical risk of TdP. This approach substantially improved prediction of torsadogenicity, compared with the Redfern method,[74] and shows how multi-factorial analysis and dry-lab integration of wet-lab data can be productive in the prediction of pharmacological (side-)effects.

Some of the most frequently used clinical biomarkers for pharmacological testing are electrocardiogram (ECG)-based (for instance the time between the onset of ventricular excitation, Q-wave, and the end of repolarization, T-wave, known as the QT interval).[87] Thus, effective computational prediction of drug risk also requires utilization of multi-cellular tissue representations,[88] so that drug actions can be computationally related to ECG changes observed in preparations from native tissue to patient. This is being addressed using simplified tissue models,[89–91] anatomically detailed whole-ventricle models,[76] and simulations of body surface potentials.[92] It is important to remember that it is essential for this approach that computational and experimental models are appropriately matched, to ensure 'like-for-like' comparison. For instance, 2D models may be best paired with cardiac tissue slices,[93] simple 3D models with coronary-perfused ventricular wedge preparations,[94] or whole-heart models with the isolated Langendorff-perfused heart,[95] and so on.

Importantly, multi-cellular models allow for consideration of spatial effects, such as changes in dispersion of electrophysiological properties and ionic currents, which may affect measured QT intervals. Significant effects of the mechanical environment, which may contribute to a load-dependent amplification (or attenuation) of pharmacological actions, may also be explored. For instance, it has been shown recently that ranolazine acts, at least in part, via effects on a mechano-sensitive component of the late Na^+ current,[96] re-emphasizing the need to consider MEC-mechanisms as potential therapeutic targets.[97]

3. Wet/dry reduction: from macro to micro

Thus far, we have considered the utility of an integrated wet/dry approach for projecting from molecular mechanisms of cardiac electrophysiology and their modulation by environmental factors (such as mechanical or pharmacological interventions) to cell, tissue, and organ function. The use of quantitative theoretical models allows one to adhere, in this context, to basic laws (such as conservation of mass, charge, energy, etc.), while integrating insight to explore causal interrelations in a complex system.

Vice versa, theoretical models can help in projecting from macro- to micro-scales, such as to assess the effects of 'gross' mechanical deformations of ventricular tissue on coronary blood flow,[98] energy demand,[99] or cell-level stress–strain behaviour.[100] They can also aid the process of linking whole-organ observations, such as obtained with optical mapping of functional parameters, to underlying tissue and cell behaviour,[101–104] as illustrated below. Whole-heart, or more frequently whole-ventricle or whole-atria models have allowed, for instance, examination of the importance of microstructure for normal and disturbed electrical behaviour,[105] or for clinical interventions to treat VT, such as electrical defibrillation[106] and tissue ablation.[107,108] These models are currently being expanded to include mechanical deformation, based on increasingly detailed descriptions of cardiac histo-anatomy.[109–112] In addition, they are being linked to fluid dynamics representations of normal, pathological, and device assisted pump-action.[113] The result has been a growing appreciation of the fact that 'biological emergence' works in both directions: emergent properties also arise at lower spatial levels than that of the considered system, as demonstrated next by brief examples.

3.1 Interpreting experimental results: effects of heterogeneity on optical mapping recordings

Being a non-contact method whose resolution can be adjusted 'on the fly', optical mapping of cardiac electrophysiology allows for projecting observations of cardiac function to underlying cellular behaviour. Optical mapping of the heart usually involves staining preparations with fluorophores sensitive to a specific parameter that, when excited by suitable wavelengths of light, emit fluorescent signals that report parameter changes. The use of such dyes has disadvantages, as many are cytotoxic and/or affect cell function (such as by binding, and hence buffering, ions whose concentration they report).[114] However, there is an increasingly broad range of dyes with progressively less intrusive properties,[115] which continues to improve the range and quality of mapping applications.

At the tissue and whole-organ level, optical mapping can be used to gain spatio-temporal information about trans-membrane potential dynamics and intracellular ion concentration changes. This has provided vital insight into mechanisms underlying the spread of cardiac excitation, maintenance of normal rhythm, and initiation/sustenance of arrhythmias.[116] Optical mapping data also has been pivotal for computational model development and validation. A common misconception, though, is that fluorescence, collected from an organ surface (say the ventricular epicardium) is representative of underlying activity (say membrane potentials) in the outermost tissue layer only. To stay with this example, the depth of myocardium in which voltage-sensitive dyes are excited depends on excitation wavelength (and factors such as whether the heart is blood or saline perfused), as light penetration into tissue increases with wavelength.[117–119] This means that optical mapping data are representative of behaviour in a tissue volume, whose extent is excitation wavelength-dependent. In addition, source-signals are distorted by photon scattering within the tissue, causing both transmural and lateral dispersion.[101–103] This is exacerbated by electrophysiological and structural heterogeneity,[120] which increases in pathologies, such as myocardial ischaemia.[121,122]

Ischaemia causes significant changes in AP configuration, compared with normal tissue, including decreased upstroke velocity, AP amplitude and duration, and a more positive resting membrane potential,[123] as well as changes in refractoriness.[124] In regional ischaemia, the layer of cells between oxygen-deprived tissue and normoxic myocardium, i.e. the border zone, does not display a fully ischaemic phenotype. This results in a lateral gradient between poorly and well-perfused tissue, and a transmural gradient at the epi- and endocardial surface (due to diffusion of oxygen from the environment surrounding the heart and from the blood within the ventricles). During global ischaemia, only transmural gradients will exist, which have been reported to extend over depths of $\sim$1 mm.[121,122] These gradients increase electrophysiological heterogeneity, and the likelihood of arrhythmia development.[125] In optical mapping studies, the epicardium is most commonly exposed to ambient air or perfusate, i.e. an environment with a partial pressure of oxygen that exceeds *in vivo* values, even of the well-perfused heart. Thus, as recordings represent weighted averages from a volume of tissue beneath the observation surface, the development of a transmural electrophysiological gradient in ischaemia may affect recorded signals.

To explore this, Dutta *et al.*[104] performed optical mapping of transmembrane potentials in isolated Langendorff-perfused rabbit hearts during global no-flow ischaemia, with alternating blue (shallow tissue penetration) and red (deep penetration) excitation of a ratiometric voltage-sensitive dye, using a novel single-sensor multiparametric optical mapping approach.[126] In control flow conditions, no differences were observed in AP morphologies recorded with blue and red excitation light during apical pacing. With increasing duration of no-flow ischaemia, AP upstroke velocity and AP duration decreased, and greater changes were seen in the signal obtained with red excitation light. This suggests that the development of transmural heterogeneity affects AP measurements, as deeper-penetrating light reveals a 'more ischaemic' phenotype. The difference between recorded AP properties during red and blue excitation was enhanced by increased pacing rate, until intramural re-entry of excitation was observed, resulting in sustained ventricular tachycardia (VT).

The plausibility of the above conceptual explanation to link whole-organ observations to underlying gradients in tissue- and cell-properties was quantitatively assessed in a computational study employing a 3D model of rabbit myocardium, representing electrophysiological changes during no-flow ischaemia, including transmural gradients,[127] and photon scattering effects.[101] Simulations showed similar differences in AP-related fluorescence, obtained during excitation with blue and red light. They further suggested that distortion of the optical signal in ischaemia was enhanced by greater transmural curvature of the excitation wavefront, due to increased heterogeneity. With faster pacing rates, transmural heterogeneity was intensified, eventually causing failure of electrical propagation (conduction block) in the ischaemic core, re-entry via the border zone, and VT. This was presumably due to a greater increase in post-repolarization refractoriness (a profoundly pro-arrhythmic change that occurs

rapidly, within the first few minutes of ischaemia, when refractoriness significantly outlasts AP duration)[124] in this region, compared with the border zone (as previously shown in the Langendorff-perfused porcine heart).[128] The hypothesis regarding underlying mechanisms remains to be experimentally assessed.

The computational results described here reinforce the need to consider effects of excitation wavelength on signals measured by optical mapping, as previously highlighted in the context of structure-based heterogeneity.[129] Moreover, combined computational and experimental exploration of multi-wavelength mapping data may help in quantifying the extent of spatial electrophysiological heterogeneity, and in exploring its role in the initiation and sustenance of arrhythmias.

It should be noted, though, that this (and most other cardiac voltage mapping) research was conducted in mechanically immobilized, or chemically uncoupled, tissue. However, cardiac function is inseparable from motion. There are abundant feedback mechanisms from mechanics to electrical activity,[27] whose elimination can affect observed results. Also, many approaches for removal of 'motion artefacts' affect electrophysiology directly (including the most commonly used electro-mechanical uncouplers, blebbistatin, and 2,3-butanedione 2-monoxime, BDM).[130] Improved structure-based representations of dynamic cardiac electrical and mechanical behaviour may help to address these limitations of current experimental techniques.

3.2 Towards patient-specific models: role of cardiac micro-structure in electrical function

Non-invasive high-resolution imaging techniques, such as magnetic resonance imaging (MRI), are providing unprecedented detail regarding the 3D structural complexity of the heart. While, generally, experimentally ill accessible, the role that heterogeneous micro-structure plays in normal and pathological ventricular electrical activity can be investigated with computational simulations of whole-ventricle electrophysiology. This approach is based on micro-scale resolution meshes, generated from MRI data, which include fine anatomical features such as the intricate network of trabeculations running along the ventricular endocardium, and coronary blood vessels within the myocardium.

A recent study by Bishop *et al.*[105] compared simulations performed using a highly detailed finite-element model of rabbit ventricles, constructed from high-resolution MRI data, with simulations using a simplified version of the same anatomy that excluded small-scale features (i.e. the coronary vessels and endocardial structures). Their study demonstrated that the presence of trabeculations may provide accessory pathways for excitation, causing regional differences in pacing-induced activation patterns between the models. With electrical field stimulation, as is applied during electrical defibrillation, these structures, as well as intramural blood vessels, may further give rise to small virtual electrodes (regional driving force for a change in transmembrane potential),[131] which can result in the genesis of wave-fronts not present in simplified models. A similar dependence of defibrillation outcome on tissue micro-structure has been shown by Rantner *et al.*[106] using a model of rabbit ventricles with chronic myocardial infarction, constructed from MRI-data and enriched with information from optical mapping experiments. Here, the upper limit of vulnerability (the weakest electrical shock that does not result in VF when applied during the vulnerable window, providing an estimate of the minimum shock-strength required for reliable defibrillation) was twice as high in the infarction model, compared with the control. In their model, the presence of a peri-infarct zone (electrically and structurally remodelled tissue) was responsible for the increased vulnerability. This was a result of less pronounced virtual electrodes in the infarction model, and delayed propagation in the peri-infarct tissue. These results illustrate the relevance of small-scale anatomical features in the genesis and termination of ventricular arrhythmias.

Structural considerations are also important for the design and optimization of anti-arrhythmic interventions, such as ablation of re-entrant circuits or focal excitations responsible for ventricular tachy-arrhythmias. Determining optimal ablation sites remains challenging, so new methods for predicting patient-specific targets may offer therapeutic advantages, both in terms of increasing success rates and reducing intervention duration. Using whole-ventricle computational models developed from individual MRI data, simulations have successfully predicted outcomes of VT ablation in infarcted pig models,[107] and patients.[108] The optimal lesion size identified computationally was consistently smaller than that created *in situ*, suggesting room for procedural improvements.

These results demonstrate that using a combined wet/dry approach to integrate relevant aspects of patient-specific data, it may be possible to guide or enhance clinical interventions.[132]

3.3 Form and function: importance of myocardial arrangement to mechanical function

Left ventricular (LV) mechanical function is determined by passive and active myocardial mechanical properties and by 3D structure and geometry (which also affects electrical properties), as reviewed elsewhere.[133] Briefly, at the micro-scale, myocyte orientation (generally referred to as 'fibre direction', even though cardiac muscle is not comprised of 'muscle fibres') is organized in healthy myocardium into a well-defined, smoothly varying transmural distribution (which, in larger mammals, varies from about -60° with respect to the chamber-horizontal plane in the epicardium, to around $+60^\circ$ in the endocardium). Myocytes are further laterally re-enforced locally into laminar layers ('sheets', though as they are not continuous the term 'sheetlet' may be more appropriate). Sheetlets are four to six cells thick and contain tightly coupled myocytes. Adjacent layers are separated by clefts, called cleavage planes, allowing local tissue rearrangement by sheetlet shearing. At the same time, longitudinal connective tissue cords, distributed throughout the myocardium, limit passive (over-)extension along the myocyte axis.

This structural organization (transmurally varying myocyte orientation, shearing of sheets, prevention of over-extension), along with LV ellipsoidal geometry and torsional deformation, results in efficient LV ejection, in spite of the restricted range of sarcomere lengths over which cardiomyocytes develop force and shorten. Yet, how exactly normal LV mechanical function is linked to deformations of the underlying anatomical structure remains unclear. This is difficult to assess experimentally, as same-heart observations of histo-anatomical detail in more than one deformation state are fraught with technical challenges (one cannot do histology on the same heart twice, and diffusion tensor MRI has limited resolution). However, using anatomically detailed, whole-LV, electro-mechanical

computational representations, it is possible to assess plausibility of conceptual explanations.

State-of-the-art models are based on large deformation elasticity theory, including representations of mechanical properties related to tissue micro-structure by incorporating spatial fibre orientation distributions, derived from standard and diffusion tensor MRI.[109–112] It has been shown that many of these models are able to reproduce LV torsion and reduction in chamber volume, but other aspects of normal LV contraction, such as the predominantly centripetal wall thickening (to ~140%), and the significant base-to-apex shortening that is essential for the heart's ability to act as a pressure/suction pump *in vivo*, tend to be captured less well.

One reason for present model limitations is that myocyte shortening and thickening alone can explain only about 20% of observed wall thickening.[133] Likewise, even in combination with physiologically plausible ranges of fibre angles, this does not necessarily give rise to physiological levels of baso-apical shortening (which sometimes is resolved by addition of baso-apically directed sub-endocardial muscle fibres, running at 90° relative to the chamber-horizontal plane). One missing aspect of LV deformation may be the inappropriately modelled laminar architecture. Unlike cell orientation, myocardial sheets are not aligned in a uniform or homogeneously changing pattern throughout the LV wall.[134–136] They represent locally prevailing arrangements of lateral cell integration, and they show acute changes in direction. It has been shown by high-resolution contrast-enhanced MRI,[137] as well as by standard and diffusion tensor MRI in combination with 3D histology,[138] that sheets are arranged in a complex fashion, forming V, N, M, or X-like patterns in transmural cross-sections. Previously, changes in laminar orientation during contraction had been implied from observations on fixed hearts, with different hearts used to study systole and diastole.[139] A recent study by Hales *et al.*,[138] using live-tissue diffusion tensor MRI of the same heart in two different mechanical states, has reported sheet re-orientation into more chamber-horizontal planes during ventricular contraction. Such laminar re-arrangement that involves a systematic increase in (apparent) sheet-intersection angles may support wall thickening and apex–base shortening, and importantly, remains consistent with the previously suggested 'sliding sheet' hypothesis.

Due to a lack of sheet orientation data, current whole-LV models generally introduce laminar orientation (if at all) by means of locally defined tensors representing electrical (conductivity) or mechanical (stiffness) parameters in three orthogonal directions (fibre, cross-fibre-in-sheet, and sheet-normal).[140] Moreover, as models employ a constant 'average' material law that bundles myocytes and the extra-cellular matrix together, there is no direct means of establishing a straight correspondence between function at macro-, meso-, and microscopic levels.

Appropriate modelling of the role of sheets in cardiac contraction may be a missing link to understanding the dynamics of LV wall thickening and atrioventricular valve-plane shift. If so, it is not surprising that 'fine-tuning' of parameters such as the stiffness tensor[140] or myocyte orientation[141] has only been partially successful in matching model predictions and patient data. Thus, a fundamentally new way of simulating presence and mechanical effects of sheets may be required to quantitatively explore the relationship between myocardial microstructure and whole-organ function. This highlights the importance of including, in computational models, all necessary aspects relevant for addressing a given problem, in spite of the drive towards simplification. It also reiterates how computational models can be exceptionally useful for gaining insight even though, or because, they may fail in reproducing certain facets of reality.

4. Outlook

Computational models represent powerful tools for the exploration of cardiac structure and function across multiple levels of spatio-temporal complexity. Solid experimental/clinical data input, and a close interaction between wet and dry research, are essential for their development, validation, and appropriate use. As demonstrated in the examples above, this results in an iterative spiral (*Figure 1*), in which mismatch between model prediction and experimental validation identifies directions for subsequent development. This may give rise to models of increased complexity, followed by model refinement and (re-)simplification, to reach the required levels of confidence, eventually moving towards models with a useful balance of simplicity/complexity and trust.

As computational models of the heart continue to be developed, incorporating progressively lower order sub-cellular components,[21,142,143] additional relevant factors (age, sex, disease states, etc.) determine their utility for interpreting individual experimental or clinical observations, as well as for predicting outcomes.[18] In certain domains, computational modelling requirements call for improved data acquisition and experimental design approaches (driven, for example, by the need for complete dose-response curve characterization, or species-specific values of biological parameters). This will help to reduce ill-controlled 'parameter inheritance', a contributor to inter-model inconsistencies.[144]

Improvements in model building can benefit from close (pre-competitive) academic-industrial and inter-disciplinary collaboration.[145] Related to this is the need for better access to, and consistency of, experimental data and meta-data. Specifically needed are: (i) good-quality data, including raw, rather than pre-analysed data sets, to allow later extraction of additional parameters; (ii) data from studies that suggest a lack of effect, to allow testing model specificity ('negative' data are useful); and (iii) standardized reporting guidelines, as experimental methods vary greatly between (and even within) laboratories, and will change over time as new technologies become available (an example of such a guideline is the recently proposed *Minimum Information for Cardiac Electrophysiology Experiments*, MICEE).[146] Access to available data and models, and adherence to effective reporting, could be fostered by a shift in academic merit-assessment systems, to encourage sharing of information, tools (models), and materials by recognizing and rewarding related activities. Additionally, a greater emphasis should be placed on multi-disciplinary training, which, while it takes longer, is utterly necessary for effective wet/dry research integration. This is especially important at a time when undergraduate training is increasingly becoming more fractionated, and where early career progression may benefit from (overly) narrow specialization.

Associated with integrative research approaches is the further promotion of translational medicine, facilitating the transition of scientific discovery from bench to bedside, as well as reverse translation, where successful medical practice becomes a subject of targeted basic research into underlying mechanisms. These important directions are not, of course, replacements of blue-skies basic research, without which innovations such as the ECG, MRI, or patch clamp would not exist in the first place.

Combined wet/dry research has been at the core of trial-and-error approaches to basic and applied biomedical research, where observation has guided conceptual development, prompting further exploration and observation. The advantage we have today is that conceptual models can be quantitatively assessed through integrated experiments using experimental and computational models. By re-developing the classic skill to observe, and combining this with quantitative modelling, the speed of biomedical research and development can be improved, increasing the efficiency of translating findings from pipette to patient.

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