CARDIAC BIOENGINEERING

More people worldwide pass away from cardiovascular disease (CVD) than from any other disease. The cardiovascular system is an incredibly complex organ system made up of the heart and blood vessels, which represent many connected, constantly moving tissue components. Heart attack, heart failure, valve failure, arrhythmia, stroke, and cardiomyopathies are only a few of the many illnesses that make up cardiovascular disease (CVD). Although there are numerous risk factors for CVD, age-related artery hardening (arteriosclerosis) and plaque accumulation inside the arteries (atherosclerosis) are understood to be the fundamental causes. Unfortunately, the methods used for CVD diagnosis, follow-up, and management are largely insufficient. Despite the paradigm shift in clinical care from an experience-based to an evidence-based approach, the field is still hampered by the large amount of data required for more effective prevention, risk stratification, or care, as well as the complex interrelationships of numerous different factors determining the ultimate fate of a given pathology. It is crucial that we continue to create new technologies and better understanding in order to combat what is one of the leading causes of death in the world due to its rapid increase. This is because there are several comorbidities that affect the aetiology and development of CVD, as well as the substantial molecular and physiological complexity.

Since its debut, APL Bioengineering has focused on seeking submissions for special issues and collections that attempt to draw attention to pressing medical and health issues and show how bioengineering breakthroughs are addressing these difficulties. In this Editorial, we highlight recent publications in APL Bioengineering that help us understand human cardiovascular function and dysfunction, the effects of ageing on the functioning of this complex system, how we can mimic aspects of human biology and pathology with in vitro cardiac tissue models, and how drug and therapeutic discovery with new human cell-based drug screening platforms will allow us to address heart and, more broadly, cardiovagal diseases. This collection, which covers a wide range of issues, includes contributions from eminent bioengineering experts in the domains of cardiac modelling, cardiac physiology and biology, animal models of heart illness, and cardiac tissue models.

Three modelling papers from the "Bioengineering of the Heart" collection provide fresh insight into right heart remodelling after pulmonary arterial hypertension, the application of artificial intelligence (AI) to more accurately map myocardial electrical activity, and the combination of clinical data to more accurately simulate large artery flow. These modelling papers are supplemented by two papers describing state-of-the-art animal models that provide novel insights into the pathology of early dissecting abdominal aortic aneurysm formation in apolipoprotein E-deficient mice and into cardiomyocyte (CM) remodelling with age by examining explanted Drosophila hearts. The collection also contains six papers that describe novel cell-based microfluidic platforms to more precisely model ischemia-reperfusion injury (IRI) following coronary intervention using induced myocardial infarction (IMI), as well as a brief review and perspective. These publications include detailed state-of-the-art views on our progress towards replicating human heart tissue and function in ex vivo models to understand disease pathways.

**Modelling Cardiac Function for Better Treatment and Prevention**

In the past few decades, cardiac modelling has developed quickly, offering increasingly realistic and representative virtual phantoms to study heart illnesses and associated treatments. The era of "patient specific" modelling, which combines imaging with clinical data of physical, mechanical, chemical, and electrical cues, enables the creation of customised, realistic morphological models. The two goals are to obtain personalised medicine paradigm indications for the diagnosis and treatment of the unique patient and to provide virtual patient populations for in silico studies. The prediction of tissue remodelling, the real-time accurate mapping of 4D physio-pathological variables from direct measurements, and the realisation of ever more trustworthy and realistic models are only a few of the technical hurdles in this scenario.

A novel structurally based constitutive model for the myocardium of the right ventricular free wall is presented by Avazmohammadi and colleagues. Their goal is to look at how pulmonary arterial hypertension affects the transmural remodelling of the right ventricular myocardium. Indeed, with a mortality rate of 37.2% at 3 years after diagnosis, right ventricular failure is a significant cause of death for individuals with pulmonary arterial hypertension. The separation of the tissue-level impacts of the mechanical and structural adaptations of myo- and collagen fibres, as well as their interactions, is made possible by their method. Their long-term objective is to investigate the potential existence of a "no return" point along the progression of hypertrophy and remodelling, beyond which the adaptive mechanisms fail to restore the wall stress value, as well as the inclusion of sophisticated and validated models of tissue remodelling in patient specific organ-level simulations for the best diagnosis, new research suggests.

In order to accurately diagnose various cardiac arrhythmias, such as premature ventricular contractions, ventricular tachycardia, atrial flutter, and atrial fibrillation, Rajagopal and colleagues present a new model for computing electrocardiogram (ECG) mapping based on a polynomial neural network. Conventional ECG recordings can pick up arrhythmia symptoms, but interpretation and location of, say, atrial fibrillation sources require extensive clinical knowledge. In this situation, anatomical x-ray computed tomography and non-invasive multi-lead body-surface ECG can be used to provide complete 3D reconstructions of heart electrical activity. In contrast to current mapping techniques, the authors of the research suggest an approach that can offer improved spatiotemporal resolution and reconstruction accuracy. Their goal is to give doctors a solid and trustworthy time-resolved 3D cardiac map of a patient before surgery so they can better understand where the patient's atrial fibrillation or premature ventricle contractions are coming from, assess whether an ablation procedure would be a good course of action, and track the patient's electrophysiology over time and while engaging in regular exercise.

Pirola and colleagues look into the validity of existing methods for simulating blood flow in the ascending aorta in the presence of aberrant fluid dynamics, such as those caused by a mechanical or stenotic aortic valve. Although it is widely acknowledged that hemodynamics plays a crucial role in vascular health, accurate in vivo quantification of important hemodynamic parameters like wall shear stresses is still not feasible. Image-based computational fluid dynamics (CFD) is the only method available for studying aortic hemodynamics. In this beautiful paper, the authors show how adding an artificial boundary constraint can result in approximations that are too close to the truth and unrealistic outcomes across the board. They compare the effects of the flat profile, a 1D through-plane velocity obtained using the phase-contrast magnetic resonance imaging technique, and the full 3D phase-contrast magnetic resonance imaging-derived velocity profiles acquired at the valve outlet on the ascending aorta fluid dynamics in particular. Results by Pirola et al. demonstrate unequivocally that when the wrong boundary condition is selected, peak and mean velocities at the proximal end of the ascending aorta can be understated by as much as 41% when the secondary flow components were disregarded.

**Creating animal models of cardiovascular disease**

In order to obtain pertinent and distinctive information to guide disease onset and tissue remodelling, an appropriate design approach can be very successful in animal model research. Animal models provide a very efficient way to quickly get comprehensive data on a variety of diseases that are challenging to obtain even through big patient cohorts and clinical research. The findings, while not directly applicable to humans, are crucial for understanding the underlying causes of diseases and how they spread in order to better understand these conditions and find new therapeutic targets.

Using apolipoprotein E-deficient mice, Phillips and colleagues explored the early pathophysiology of dissecting abdominal aortic aneurysm formation at various stages by continuously administering angiotensin II. Because many patients with dissecting abdominal aortic aneurysms have aneurysms with sizes less than 5 cm, preventing surgical treatment, this research represents a substantial advancement. Considering this, it should be possible to identify high-risk patients who would benefit from treating smaller aneurysms by understanding how early abdominal aortic aneurysms form and grow. To find the emergence of abdominal aortic aneurysms and aortic dissections, the researchers adopted a daily ultrasonography screening approach. The collection of information from RNA sequencing, gene expression analysis, histology, and immunohistochemistry was added to this approach. Phillips et al. measured extracellular matrix remodelling and inflammatory cell infiltration within 24 hours of aortic dissection or at 10 days after angiotensin II infusion in order to gain new insight into the biomechanical, microstructural, and inflammatory changes occurring in mice with and without aortic dissection.

The extracellular matrix and intracellular components of the heart undergo substantial remodelling throughout life, which reduces contractile compliance and elasticity. The thickening of the left ventricular wall, an increase in systolic pressure, and reduced myocardial function are all related occurrences. Sessions and colleagues looked into how the upregulation of cardiac vinculin might function as a compensatory mechanism during ageing. They discovered a compensatory mechanism in which vinculin-mediated cytoskeletal reinforcement enhances force production and myofibril resistance balances age-related increases in heart wall strain. The scientists demonstrated that cardiac Vinculin overexpression with ageing is a conserved process in non-human monkey, rat, and fly models, independent of cardiovascular illness, using a multi-model approach. They specifically took beating hearts from Drosophila melanogaster, a fruit fly, and looked at them to statistically explain the alterations in myofiber architecture. They showed that over-expression of the fly heart-specific vinculin enhances contractility, maintaining cardiac respiration with ageing or under mechanical stress. Additionally, they saw that organismal fitness rose with age, in part because glucose was oxidised by aerobic means more effectively. Overall, the ability of cardiac tissue cells to withstand mitochondrial stress and sustain rhythmic contraction may be underpinned by cytoskeletal strengthening. These findings offer important new insight into heart ageing and potential targets for future therapies because they are the first to show that cardiac-restricted cytoskeletal remodelling causes a systemic metabolic response.

**In-Vitro Bioengineered Heart Models**

A review and a viewpoint open the collection of works on in vitro created heart tissue models. Callaghan et al.'s thorough and thorough review evaluates our capacity to model cardiac complexity, providing a suitable biological and regulatory framework for bioengineers interested in contributing their knowledge to numerous aspects of cardiac bioengineering, drug toxicity, and therapeutic drug discovery, as well as outlining the status (and restrictions) of current physiological measurement modalities and techniques that can be used by biologists. The authors provide an up-to-date assessment of the significant advancements and opportunities brought about by modern experimental methodologies and strategies, discussing novel single and multicellular models and their utility and efficacy, the significance of simulating physiological states and cues (extracellular, intracellular, mechanical, and electrical), disease models and their viability, and the relevance of tissue engineered constructs and their capacity to model physical processes. Importantly, they emphasise that even though new biomimetic models and improved functional assays are crucial components of the workflow for better pharmaceutical testing and clinical outcomes and represent significant steps towards advancing our fundamental knowledge through translational research, they suggest that these are only the beginning of what may be feasible, which is an exciting proposition for the field.

The development of hPSC-CM (and cardiac tissue) is the subject of the Perspective paper by Mills and Hudson7, which focuses on what has been accomplished and what needs to be done. hPSC-CMs have been successfully employed to study hypertrophy, electrophysiology, medication toxicity and discovery, and basic biology thanks to decades of research that resulted in their creation. Since adult human heart maturity has not yet been attained and the molecular pathways of cardiac maturation are still unknown, applicability and translatability are, nevertheless, still limited. The authors provide an overview of the most suitable characterization tests and maturation biomarkers as well as a review of the most promising methods for driving hPSC-CM maturation. Particular focus is placed on the recent successes brought about by the addition of multicellularity, mechanical stress, and pacing; metabolism is also introduced as a key factor in maturation. In order to better understand the still poorly understood maturation process of the heart, as well as to produce better in vitro models for many bioengineering and therapeutic discovery applications, better methodologies to produce fully mature "adult" hPSC-CMs are clearly illustrated by the authors in this perspective's conclusion.

Cardiomyocytes (CMs) produced from induced pluripotent stem cells were used in a unique in vitro model of ischemia-reperfusion injury (IRI) that was described by Hidalgo et al. This study highlights both the advantages of using metabolically matured human iPSC-derived cardiomyocytes (which develop in just 8 days in their medium) and the limitations of earlier attempts to simulate the critical physiological state changes that take place in vivo after an ischemia attack. While all previous ischemia models remove glucose from the media during the ischemic episode, they show that, in contrast, glucose (which mimics glycogen stores in resident CMs) must be available (to prevent the onset of cardio-protective autophagy) and the pH must be lowered during the ischemic episode (to pH 6.2 to recreate local acidification) in order to mimic the known transient changes in the interstitial tissue microenvironment during an IRI event in vivo. They reproduce the observed in vivo levels of CM death (60%) following an ischemic-reoxygenation episode in vitro using this model. By testing their model against known pharmacological post-conditioning (PPC) medication candidates, they are able to validate that the observed reduction in reperfusion-induced CM cell death was consistent with the results of clinical trials. This straightforward but beautiful in vitro human iPSC model presents a novel method for investigating IRI and validating and screening human-specific PPC medication candidates.

Visone et al. created a distinct and straightforward in vitro cardiac tissue model using a microbioreactor that combines biochemical, mechanical, and electrical stimuli—important cardio-physiological signals that affect heart cell fate and development. They replicated parts of the intricate electro-mechanical environment of the heart by giving "in-device" created 3D cardiac microtissues a homogeneous electric field and cyclic uniaxial strains. The utility of this novel platform and the significance of multiplexing the pertinent stimuli ex vivo to mimic in vivo tissue states were confirmed by the controlled application of both a low voltage electric field and mechanical stretch (10% strain), which led to significant improvements in cardiac tissue maturity and function. Human cardiac cell types, including iPSC-derived tissues, can be easily exploited thanks to the flexibility of the developed microfluidic platform, which allows for the introduction of any cell type (singly or in combination) and soluble factors within input streams. This makes it possible to screen therapeutics on truly functional 3D cardiac microtissues.

Gonzalez Rodriguez et al. described a prospective treatment approach to valvular heart disease, primarily fibrotic aortic valve stenosis (FAVS), another developing cardiovascular (cardiac-specific) condition, in accordance with our more numerical publications in this collection. Without new treatments, it is expected that the prevalence of valvular heart disease (VHD) would double by 2050 due to an ageing population (much like heart failure). The only method of treatment now available is valve replacement surgery. Gonzalez Rodriguez et al.'s contribution described a hydrogel-based 3D culture method for the carefully regulated distribution of FGF-2 and TGF-B, two of the well-known major effectors of cellular transformation and fibrotic matrix deposition, to valvular interstitial cells (VICs). Similar to the various fibroblast populations in the heart, these cells maintain the extracellular matrix in heart valve leaflets, but they also function as wound-healing cells in the event of injury. This study highlights the importance of physiologically relevant 3D microenvironments in maintaining a quiescent VIC phenotype before exposure to cytokines, which transform them into myofibroblasts, the observation of matrix contraction (or lack thereof), and the use of peptide-functionalized, matrix metalloproteinase (MMP)-degradable poly(ethylene glycol) (PEG) hydrogels that recapitulated key biochemical and biomechanical valve leaflet microenvironments. Comparisons between the responses of VICs encapsulated in these hydrogels and VICs in porcine aortic valve explants provided an essential validation of the potential of this in vitro model for rapid translation to therapeutic screening approaches and also to further understand the initiation phases of this and other fibrosis-related diseases. These results confirm that exogenously administered variables have similar effects on explanted tissues.

Menon et al. described a unique microdevice to study vascular inflammation and leukocyte-endothelial interactions in 3D artery stenosis while staying with dysregulated (chronic) inflammation and wound healing. This method has a direct impact on our understanding of atherosclerosis, a key cause of CVDs such acute myocardial infarction (heart attack). This disease mostly damages the vascular bifurcation due to the accumulation of cholesterol-containing low-density lipoproteins in the sub-endothelial space in these regions. These authors present a novel, pneumatically actuated 3D stenosis blood vessel model that addresses previous shortcomings of other models by enabling tunable 3D constrictions within their cell-laden vessel-like channel to mimic the impacts of stenotic plaque inclusions and the resulting changes in hemodynamics, stresses at cell surfaces, and cell adhesion under flow of multiple (sequentially exposed and relevant to disease progression, i.e., leukocytes) cell types. Validation of full healthy blood/liquid biopsies and inflammatory cytokines (with or without these cytokines)-induced inflammation and endothelial dysfunction-induced cell attachment within the device indicated the technology's substantial potential for adoption in Point-of-Care (POC) testing of patients to stratify atherosclerosis susceptibility and high throughput screening tests for drug discovery.

The Bioengineering of the Heart collection offers three different and complimentary methods for modelling cardiac disease, each of which offers a particular perspective on heart function and repair. This anthology contains essays by experts pertaining to bioengineering serves as a convincing example of how the right combination of an engineering mindset and a thorough understanding of biology and physiology can offer previously unheard-of design and technological opportunities to assess heart function and disease and find novel therapeutic pathways to achieve functional repair of the cardiovascular system.

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