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Bioengineering of the heart

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The cardiovascular system is an extremely complex organ system made up of the heart and blood vessels, representing many connected, constantly moving tissue components that give rise to a multitude of cellular and tissue level dysfunctions and diseases—more people die of cardiovascular disease (CVD) than any other disease globally. Cardiovascular disease (CVD) represents a broad range of interrelated disorders, including hypertension, heart attack and failure, valve failure, arrhythmia, stroke, and cardiomyopathies. While there are many drivers of CVD, age-related hardening of (arteriosclerosis) and plaque buildup within (atherosclerosis) the arteries are recognized as the underlying causes. Strategies for diagnosis, follow up, and care associated with CVD are unfortunately largely inadequate. Despite the paradigmatic shift from an experienced-based approach to an evidence-based approach in clinical care, the field remains hampered by the substantial amount of data needed for more effective prevention, risk stratification or care, and the complex interrelationships of multifarious different factors determining the ultimate fate of a given pathology. Given the substantial biological and physiological complexity and the range of comorbidities that contribute to the etiology and progression of CVD, it is vital that we continue to develop better understanding and novel technologies to address what is one of the fastest growing causes of death worldwide.

APL Bioengineering, since its inception, has focused on inviting contributions to special issues and collections that aim to highlight major medical and health challenges and exemplify how bioengineering innovations are addressing these challenges. In this Editorial, we highlight the contributions recently published in APL Bioengineering aimed at providing better understanding of the human cardiovascular function and dysfunction, the impacts of aging on the function of this complex system, how we may 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 enable us to address heart, and more broadly, cardiovascular disease head-on. This collection represents a broad range of contributions by leading bioengineering experts in the areas of cardiac modeling, cardiac physiology and biology, animal models of cardiac disease, and cardiac tissue models.

The "Bioengineering of the Heart" collection includes three modeling papers, which offer new insight into right heart remodeling subsequent to pulmonary arterial hypertension,[1] the use of artificial intelligence (AI) for more effective mapping of myocardium electrical activity,[2] and the merger of clinical data for more realistic flow simulations in large arteries.[3] These modeling papers are complemented by two papers describing state-of-the art animal models that provide new insights into early pathology of dissecting abdominal aortic aneurysm formation in apolipoprotein E-deficient mice[4] and into cardiomyocyte (CM) remodeling with age by studying explanted Drosophila hearts.[5] The collection also includes six papers, including a concise review[6] and a perspective,[7] which give comprehensive state-of-the-art positions on how close we are to mimicking human heart tissue and function so as to unravel disease mechanisms using *ex vivo* models, complemented by a number of original research articles that detail novel cell-based microfluidic platforms to more effectively model ischemia-reperfusion injury (IRI) following coronary intervention using induced Pluripotent Stem Cell (iPSC)-derived cardiac tissues,[8] the electrical activity of cardiac microtissues,[9] the interplay of endothelial cells and blood cells in stenoses,[10] and the role of valvular interstitial cells (VICs) in wound healing.[11]

MODELING CARDIAC FUNCTION FOR BETTER DIAGNOSIS AND PREVENTION

Cardiac modeling has rapidly evolved in the last few decades providing progressively more realistic and representative virtual phantoms

to investigate heart pathologies and their corrections. We are now in the "patient specific" modeling era, where imaging is used to create realistic and customized morphological models, complemented with clinical data of physical, mechanical, chemical, and electrical cues. The objective is twofold: attain indications for the diagnosis and the therapy of the specific patient according to the personalized medicine paradigm and make available virtual patient populations for *in silico* trials. In this scenario, technical challenges are manifold and include the prediction of tissue remodeling, the real time accurate mappings of 4D physio-pathological quantities from direct measurements, and the realization of even more reliable and realistic models.

Avazmohammadi and colleagues[1] present a novel structurally based constitutive model for the myocardium of the right ventricle free wall. Their objective is to investigate the transmural remodeling of right ventricular myocardium in response to pulmonary arterial hypertension. Indeed, right ventricular failure is a major cause of mortality for patients suffering from pulmonary arterial hypertension, with a mortality rate of 37.2% at 3 years post-diagnosis. Their approach allows for the separation of the tissue-level effects of the mechanical and structural adaptations of myo- and collagen fibers and the interaction between them. By studying the transmural variation in adaptations of fiber orientation distribution and recruitment, their long term goal is the investigation of the possible existence of a "no return" point along the hypertrophy and remodeling progression, beyond which the adaptive mechanisms fail to restore the wall stress value, as well as the inclusion of sophisticated and validated models of tissue remodeling in patient specific organ-level simulations for optimal diagnosis, new individualized interventions and treatment protocols for pulmonary artery hypertension.

Rajagopal and colleagues[2] present a new model for computing electrocardiogram (ECG) mapping based on a polynomial neural network for the accurate diagnosis of various types of cardiac arrhythmias including premature ventricular contractions, ventricular tachycardia, atrial flutter, and atrial fibrillation. Although signs of arrhythmias can be detected with conventional electrocardiogram recordings, interpretation and localization of, for example, atrial fibrillation sources require significant clinical expertise. In this context, electrocardiographic imaging can provide full 3D reconstructions of heart electrical activity from non-invasive multi-lead body-surface ECG and anatomical x-ray computed tomography images. In their paper, the authors propose an algorithm able to provide enhanced spatiotemporal resolution and reconstruction accuracy with respect to current mapping methods. Their aim is to provide clinicians with a robust and reliable time-resolved 3D cardiac map of a patient prior to surgery to improve patient outcomes—by supporting the localization of dominant sources of atrial fibrillation or premature ventricle contractions, to determine whether an ablation procedure would be an effective treatment, and to monitor patients' electrophysiology conditions over time and during regular physical activity.

Pirola and colleagues[3] investigate the reliability of current approaches for the simulation of blood flow in the ascending aorta in the case of abnormal fluid dynamics, as for a stenotic aortic valve or a mechanical valve. Despite the fact that the role of hemodynamics in vascular health is well recognized, the accurate *in vivo* quantification of key hemodynamic parameters such as wall shear stresses is still unfeasible—image-based computational fluid dynamics (CFD) represents the sole approach for the study of aortic hemodynamics. In this elegant paper, the authors demonstrate that imposing an unrealistic boundary condition can lead to rough approximation and unrealistic results in the whole domain. In particular, they compare the effect of three different inlet boundary conditions on the ascending aorta fluid dynamics, namely, the flat profile, a 1D through-plane velocity obtained with the phase-contrast magnetic resonance imaging technique, and the full 3D phase-contrast magnetic resonance imaging-derived velocity profiles acquired at the valve outlet. Results presented by Pirola *et al.* clearly show that when the incorrect boundary condition is chosen, peak and mean velocities at the proximal end of the ascending aorta can be underestimated by up to 41% when the secondary flow components were neglected.

ENGINEERING ANIMAL MODELS OF CARDIOVASCULAR DISEASE (CVD)

A proper design approach can be extremely effective in animal model studies in order to extract relevant and unique information to inform disease onset and tissue remodeling. Animal models offer a very effective path to the collection of detailed data in a limited time frame on specific diseases that are difficult to attain even from large patient cohorts and clinical studies. The results, although not directly transferrable to humans, are pivotal to unravel the underlining disease mechanisms and their progression, with the final goal to improve the disease knowledge and identify new therapeutic targets.

Phillips and colleagues[4] have investigated the early pathology of dissecting abdominal aortic aneurysm formation at multiple scales in apolipoprotein E-deficient mice by continuously infusing angiotensin II. This work represents an important innovation, since a consistent fraction of patients affected by dissecting abdominal aortic aneurysms have an aneurysm diameter of less than 5 cm and hence do not meet the criteria for surgical intervention. Accordingly, understanding early abdominal aortic aneurysm formation and progression could assist the identification of high risk patients who would benefit from the treatment of smaller aneurysms. In their study, the authors have implemented a daily ultrasound screening approach to diagnose abdominal aortic aneurysm formation and aortic dissections, complemented by the collection of RNA sequencing data and gene expression analysis, together with histology and immunohistochemistry. Phillips *et al.* were able to evaluate extracellular matrix remodeling and inflammatory cell infiltration within 24 h from aortic dissection or at 10 days post angiotensin II infusion, providing new insight into biomechanical, microstructural, and inflammatory changes occurring in mice with and without aortic dissection.

During life, the heart remodels extensively, altering the extracellular matrix and intracellular structures that decrease elasticity and contractile compliance. Related events are the increase in the systolic pressure and afterload, left ventricular wall thickening, and impaired myocardial performance. Sessions and colleagues[5] investigated how cardiac Vinculin upregulation may act as a compensatory mechanism during aging, identifying a compensatory mechanism where Vinculin-mediated cytoskeletal reinforcement improves force production, and myofibril resistance counterbalances age-associated increases in heart wall strain. Using a multi-model approach, the authors showed that cardiac Vinculin upregulation with age is a conserved mechanism across non-human primate, rat, and fly models, independent of cardiovascular disease. In particular, they harvested and examined beating hearts of Drosophila melanogaster—the fruit fly—to quantitatively

describe the changes occurring in myofiber structures. They demonstrated that fly heart-specific Vinculin over-expression increases contractility, sustaining cardiac respiration with age or under conditions of mechanical stress. They also observed increased organismal fitness with age, in part due to more efficient aerobic oxidation of glucose. Overall, cytoskeletal reinforcement may serve as the underlying mechanism that allows cardiac tissue cells to resist mitochondrial stress and maintain rhythmic contraction. These findings are the first to document that cardiac-restricted cytoskeletal remodeling results in a systemic metabolic response, representing valuable new insight into cardiac aging and possible targets for future interventions.

BIOENGINEERED *IN VITRO* MODELS OF THE HEART

The selection of papers focused on *in vitro* engineered heart tissue models opens with a review and a perspective. In their detailed and rigorous review, Callaghan *et al.*[6] appraise our ability to model cardiac complexity, providing an appropriate biological and regulatory framework for those bioengineers interested in contributing their expertise to many facets of cardiac bioengineering, drug toxicity, and therapeutic drug discovery, while also detailing the existing status (and limitations) of current physiological measurement modalities and techniques that can be applied by biologists to decipher their complex questions surrounding cardiomyocyte biology and physiology. They provide a state-of-the-art picture of the substantial advancements and opportunities provided by recent experimental methodologies and strategies, discussing novel single and multi-cellular models and their competence and utility, the importance of mimicking physiological states and cues (extracellular, intracellular, mechanical, and electrical), disease models and their patency, the relevance of tissue engineered constructs and their ability to model physiological status (whether healthy or diseased), and the different force measurement techniques currently afforded to the field at single- and multi-cellular and tissue-levels, and outstanding needs in this particular, critical, arena. Importantly, they highlight while the creation of new biomimetic models and improved functional assays represent significant steps toward improving our basic understanding through to translational research and are critical components of the workflow to improved pharmaceutical testing and clinical outcomes; they suggest that these are just the beginning of what may be possible, an exciting proposition for the field.

The Perspective paper by Mills and Hudson[7] focuses on what has been achieved and what is still to be accomplished in the area of human pluripotent stem cell-derived cardiomyocyte, hPSC-CM (and cardiac tissue), maturation. Decades of research have led to the generation of hPSC-CMs, which have been successfully used to study hypertrophy, electrophysiology, drug toxicity and discovery, and fundamental biology. However, applications are still limited and translatability is still limited, since maturity similar to that of the adult human heart has not yet been achieved and the molecular mechanisms of cardiac maturation are still unclear. The authors review the most promising strategies to drive maturation of hPSC-CMs and offer an overview of the most appropriate characterization tests and maturation biomarkers. Specific attention is given to the recent positive results that have been achieved by the introduction of multi-cellularity and mechanical loading and pacing; metabolism is also introduced as a major driver of maturation. This Perspective closes by the authors clearly illustrating the need for better methodologies to produce fully mature "adult" hPSC-CMs, which will not only contribute to deciphering the still poorly understood maturation process of the heart, but moreover, produce better *in vitro* models for many bioengineering and therapeutic discovery applications.

Hidalgo *et al.*[8] described a novel *in vitro* model of ischemia-reperfusion injury (IRI) using induced pluripotent stem cell-derived cardiomyocytes (CMs). This study highlights previous deficiencies in our *mimicry* of important physiological state transitions that occur *in vivo* post an ischemic episode, along with the utility of using metabolically matured human iPSC-derived cardiomyocytes (taking only 8 days in their media). While all previous ischemia models *remove* glucose from the media during the ischemic episode, they show that, in contrast, in order for the known transient changes in the interstitial tissue microenvironment during an IRI event *in vivo* to be mimicked *in vitro*, glucose (mimicking glycogen stores in resident CMs) must be available (to avoid the onset of cardio-protective autophagy) and the pH must be lowered during the ischemic episode (to pH 6.2) to recreate local acidification. From this model, they recreate the observed *in vivo*, post an ischemic-reoxygenation episode, levels of CM death, *in vitro* (∼60%). Challenging their model with screens of known pharmacological post-conditioning (PPC) drug candidates, they confirm that their observed reperfusion-induced CM cell death was substantially reduced, in-line with clinical trial outcomes. This simple but elegant human iPSC-derived *in vitro* model offers a new approach to study IRI and validation and screen human-specific PPC drug candidates.

Visone *et al.*[9] described a novel and facile *in vitro* cardiac tissue model, constituting a microbioreactor in which they have combined biochemical, mechanical, and electrical stimuli, important key cardio-physiological cues driving cardiac cell fate and maturation. By providing a uniform electric field and cyclic uniaxial strains to "in-device" generated 3D cardiac microtissues, they successfully recapitulated components of the complex electro-mechanical environment of the heart. Controlled application of both a low voltage electric field and mechanical stretch (10% strain) provided substantial improvements in cardiac tissue maturity and function and confirm the utility of this novel platform and the importance of multiplexing the relevant stimuli *ex vivo* to mimic *in vivo* tissue states. The flexibility of the developed microfluidic platform to introduce any cell type (single or in combination) and soluble factors within input streams offers ease-of-exploitation to human cardiac cell types, including iPSC-derived tissues, and the screening of therapeutics on truly functional 3D cardiac microtissues.

In line with our more numerical papers in this collection, Gonzalez Rodriguez *et al.*[10] detailed a potential therapeutic approach to another growing cardiovascular (cardiac-specific) disease, valvular heart disease, principally fibrotic aortic valve stenosis (FAVS). With an aging population, by 2050, without new treatments, valvular heart disease (VHD) is predicted (much like heart failure) to double. Valve replacement surgery is the only currently available treatment. In their contribution, Gonzalez Rodriguez *et al.* described a hydrogel-based 3D culture system for the controlled delivery of two of the known primary effectors of cellular transformation and fibrotic matrix deposition, FGF-2 and TGF-B, to valvular interstitial cells (VICs). Like many fibroblast populations in the heart, these cells maintain the extracellular matrix in heart valve leaflets but are also responders for wound healing in the advent of injury. This study, by using peptide-functionalized, matrix metalloproteinase (MMP)-degradable poly(ethylene glycol)

(PEG) hydrogels that recapitulated key biochemical and biomechanical valve leaflet microenvironmental properties, highlights the importance of physiologically relevant 3D microenvironments in enabling maintenance of a quiescent VIC phenotype prior to exposure to cytokines (that transition them into myofibroblasts), the observation of matrix contraction (or absence thereof), and also, the ability of FGF-2 to mediate the fibrotic phenotype effects of TGF-B1 on these important cells in such environs. Comparisons between the responses of VICs encapsulated in these hydrogels and VICs in porcine aortic valve explants confirmed similar impacts of exogenously delivered factors in explanted tissues, providing important validation of the potential of this *in vitro* model for rapid translation to therapeutic screening approaches and also to further understanding the initiation phases of this and other fibrosis-related diseases.

Sticking with dysregulated (chronic) inflammation and wound healing, Menon *et al.*[11] described a novel microdevice to investigate vascular inflammation and leukocyte-endothelial interactions in 3D vessel stenosis. This model has direct implications in understanding atherosclerosis, a leading cause of CVDs, including acute myocardial infarction (heart attack). This disease preferentially affects vessel bifurcation and results from 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 deficiencies of other models, through 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 shear-induced endothelial dysfunction and inflammation-induced cell binding within the device when treated with inflammatory cytokines and whole healthy blood/liquid biopsies (with or without these cytokines) confirmed the utility of the device and significant potential for translation into high throughput screening assays for drug discovery and Point-of-Care (POC) testing of patients to stratify susceptibility to atherosclerosis.

The Bioengineering of the Heart collection provides three distinct and complementary approaches to cardiac disease modeling, each providing specific insight into cardiac function and repair. This collection of papers by leaders in the field of Bioengineering is a clear demonstration of the fact that a proper balance of an engineering mindset combined with in-depth biological and physiological understanding can provide unprecedented opportunities, in terms of design and technologies, to evaluate cardiac function and disease and discover new therapeutic avenues to achieve functional repair of the cardiovascular system.

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