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Architected mechanical designs in tissue engineering

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Abstract

The deeper comprehension of biological phenomena has led to the pursuit of designing and architecting complex biological systems. This has been incorporated through the advances in bioprinting of artificial organs and implants even at the microscale. In addition, tissue modeling has been employed to understand and prevent malfunctional and detrimental mechanisms that lead to fatal diseases. Furthermore, the endeavor to convey the mechanical properties of both scaffolds and cells has enabled the unveiling of disease modeling and regenerative medicine. This paper aims to provide a brief review of the design, modeling and characterization of conventional and architected structures employed in bioengineering.

Introduction

During the last decades, there has been a significant progress in the design and modeling of artificial tissue engineering. That is a repercussion of our assiduity to deeper comprehend both the cellular and extracellular matrix (ECM) behavior of such systems.[1] However, their mechanical response is evinced substantially differently for a variety of different mechanisms, such as shear stresses in blood vessels[2,3] for blood flow or tensional and compressive forces in muscles[1] for instance. In addition, the environment wherein the cells reside has an imperative role in their response.[4]

Different external stimuli can affect the development and growth of the ECM and the cells in remarkably different ways.[5,6] To this end, tissue modeling has been exponentially contemplated to provide an answer on how the combination of applied forces and the structural environment affects the cellular behavior.[7] Based on either fundamental mechanics[8] or in vitro testing,[9] the mechanical behavior of the cells can be illuminated depending on the applied external stimuli. The characteristic examples of such phenomena are the dynamic reorganization of the cytoskeleton,[10] tension-dependent assembly of the actin and myosin into stress fibers, the cross-bridge cycling between the actin and myosin filaments,[11] or even the triggering of specific internalization pathways, such as endocytosis and macropinocytosis.[1]

Furthermore, from the perspective of the design variables that can be tailored, the properties of the ECM or the scaffold, such as the rigidity[12,13] and configuration of the structural members[14] can control a plethora of different phenomena, such as stem cell intracellular signaling, membrane rearrangement, proliferation, differentiation, migration, actuation, and cell adhesion.[11,15] All of these properties can be efficiently monitored and controlled through either an ECM or a two-dimensional (2D) environment.[5,7,16] These various properties, that are critical for the operation of the cells as a constituent component or a part of a tissue or organoid, span a vast category of different cells. The characteristic examples that their response has been controlled are neurons,[17–19] U87 cells[14,20] (i.e., glioblastoma cell line), or even human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs).[21] All of these different cells and their respective responses expand our understanding of mechanobiological phenomena from neurological to cardiac systems. While the mechanical response of such systems is comprised of mechanisms occurring in the highly nonlinear domain (e.g., viscoelasticity, plasticity, and nonlinear elasticity),[1,10] fundamental constitutive models have been proposed, effectively characterizing the manifested stress and displacement fields of either the cell, the tissue, or the scaffold.[22–24]

These advances have also been accomplished by progress in multiphoton lithography (MPL).[25] MPL enables the fabrication of complex architected matrices, imitating the features of the environment that the cells can interact with each other.[21] Moreover, it can be utilized to even emulate environments that will lead to malfunctional behaviors of the tissue, such as cardiomyopathy[26] and explicate them. The realization of

these effects has been steadily employed for the design of three-dimensional (3D) tissues and organoids with the objective to design bioimplants.[27] While these architected organs will provide an avenue for implants and experimentation without living specimens, a new trend aims to create implants that surpass the conventional behavior of living organisms, scilicet meta-implants.[28]

Employing properties found in architected materials,[29] novel designs such as kirigami or origami[30] can be used as scaffolds to create implants or may lead to organs with unprecedented properties. More specifically, bistability through large deformations induced by buckling can lead to malleability and actuation of the structure.[31] In addition, auxeticity, leading to negative Poisson's ratio, provides resilience of the scaffold, impeding localized failure that inevitably leads to malfunction.[32]

All of these aspects of tissue engineering at the design level of the ECM or the cellular response are steadily convening to provide novel techniques and methodologies in artificial organ design. Figure 1 conveys all of these domains that must be convened for tissue design and biomanufacturing. In this brief review, we will address the major aspects of these domains and explore the common links between them. First, we will address the modeling and design in tissue engineering, leading to efficient ways to tailor the cellular behavior at the constitutive level or with in vitro testing. Next, we will address the progress in scaffold bioengineering, how it is utilized to imitate a healthy or a malfunctional environment and how it affects different cellular mechanisms. The control parameters that will be reported are both the cell types and the architected matrix architecture. Finally, we will explore how the design paradigm of metamaterials can be employed to improve and tailor the response of the organs. This review aims to illuminate the landscape of tissue engineering from the perspective of mechanical behavior and to set to framework for further advancement of these domains, some of which are still nascent.

Tissue design and modeling

In the literature, there are two main approaches to depict the mechanical behavior of tissues and cells under the influence of the external environment. The first is through a bio-chemo-mechanical model that provides the constitutive relation between forces and displacements during the deformation of the cell.[22–24] The second requires a patterned surface, either 2D or 3D, that living cells can be attached, enabling the observation and characterization of their behavior.[33] While the first method is predicated on simple mechanical systems to elucidate whether the constitutive relation is consonant, the second method enables the depiction of much more complex phenomena, providing more information related to the physiological response of the cells or the developed tissue.

At this point, it is instructive to provide further details regarding the mechanical response of the cells and the materials of their environment from a purely experimental perspective. The primary materials that were employed to study the impact of the ECM mechanics on the cellular function have been the hydrogels.[34] Nevertheless, in the hydrogel environment, the fibrous structural features and the compositionally constrained biological ligands are often interconnected, rendering the contribution to the cell behavior of each individual material property obscure to capture, such as the binding affinity for cells, the mechanical stiffness, the fibrous arrangement, the porosity, and viscoelasticity.[1] Therefore, novel techniques were employed to investigate the effect of stiffness, degradability, and viscoelasticity. The polyacrylamide (PAAm) gels and polydimethylsiloxane (PDMS) were utilized to study the mechanics of cell adhesion and migration.[35] The different surface chemistry of such gels enabled the control of the density of conjugated biological ligands. Regarding the stiffness of the substrate, it can be controlled by directly varying the ratio of polymer and cross-link solution, the curing temperature, and the duration of curing. Since these gels possess linear elastic behavior, decoupling biomechanical signals from the substrate stiffness can be achieved. Utilizing this finding, it was observed that the matrix rigidity can regulate the cell morphology. Cells that adhere to stiff substrates demonstrate a larger contact area and display higher proliferation in comparison to adhesion on softer substrates.[36] When the cell interacts with the ECM protein (fibronectin), a class of molecules called integrins engage with this protein, leading to morphological variations, enabling clustering and adhesion of integrins. Therefore, the cell is stable on the substrate, providing actin strands that are polymerized in the lamellipodia of the cell to migrate at the center of the cell. This mechanism is called retrograde flow[1] and causes an increasing tension that is transferred to the fibronectin fibers. When the cell is on soft substrates, this force will lead to the displacement of the ECM, without any resilience. However, on stiff substrates, the ECM fibers will resist against the probing force, generating traction and resulting in stretching and unfolding of adaptor proteins, such as talin and vinculin located. This event triggers the formation and expansion of filament actin stress fibers that permeate through the cell. This phenomenon leads to a mechanism called durotaxis, a propensity of the cells to migrate from soft to stiffer substrates. Despite the insight provided by such mechanisms, these phenomena are predicated on linear elasticity. However, as it will be shown next in the constitutive modeling, more complex phenomena must also be considered, such as plasticity and viscoelasticity. These mechanisms have significant repercussions on increased focal adhesion ligand density, enhanced adhesion signaling, cell spreading, and proliferation signaling. Therefore, these effects must also be included in the modeling of the system that will be presented next.

Regarding the bio-chemo-mechanical modeling, a constitutive model for the contractility of cells has been proposed that takes into account the dynamic reorganization of the cytoskeleton of the cell.[23] While previously employed models consider the cytoskeleton as an interlinked structure of passive filaments,[8] the biochemical effects that lead to resultant forces are neglected. This significantly constrains the realistic

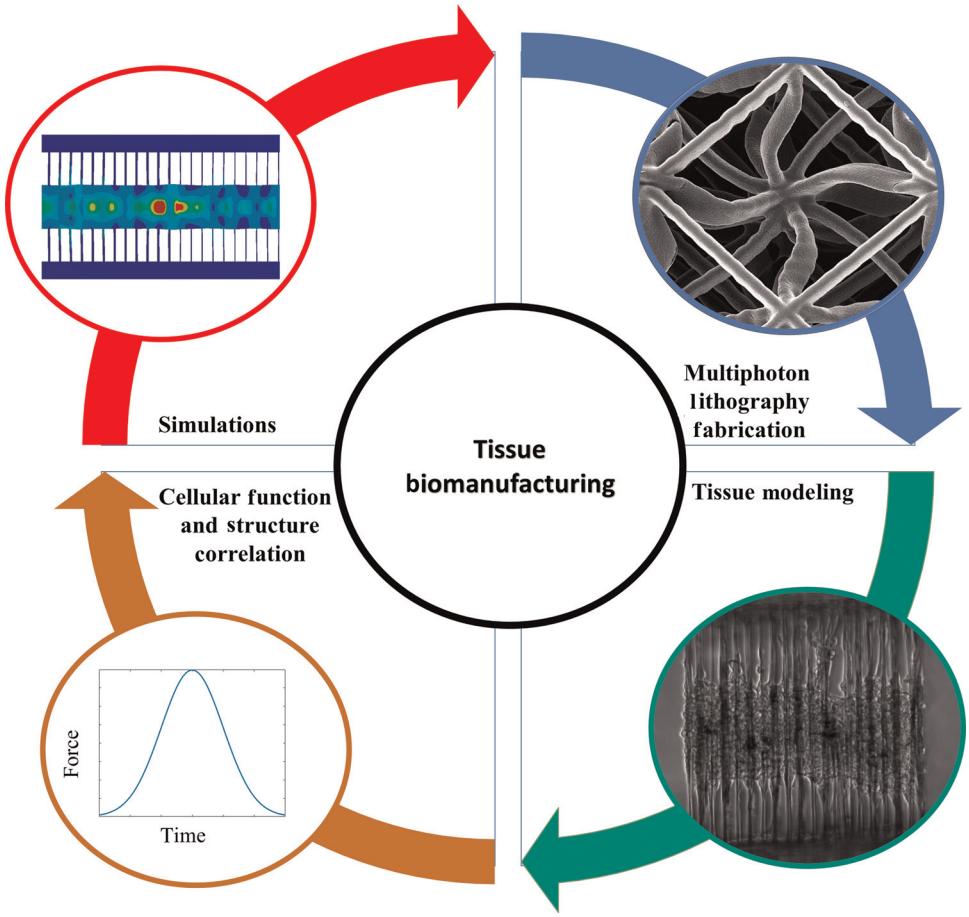

Figure 1. Schematic illustration of the design pillars of tissue biomanufacturing. By performing FEA analysis, the tissue response as a function of the scaffold geometry can be captured and tailored. Then, microscale scaffolds can be realized through multiphoton lithography. Seeding these scaffolds with cells can lead to tissue modeling and in vitro observation of the cellular response. In addition, the different cellular functions can be measured and correlated as a function of the structure geometry and be utilized as feedback to validate the model that was employed in the FEA analysis.

depiction of the mechanical behavior of the cells and the model is bereft experimental validation. However, the aforementioned suggested model employs the activation signals that inhibit actin polymerization and myosin phosphorylation, as well as the tension-dependent agglomeration of the actin and myosin into stress fibers, and the cross-bridge cycling between the actin and myosin filaments that precipitate the tensile load. This information can be utilized such that the generalized model must be capable of characterizing the fundamental interactions among the forces, the convening and dissolution of stress fibers, and also the compliance of the substrate. Even though a lot of imperative details regarding the biochemical processes that occur during the deformation of the cell have yet to be unraveled with veracity,[11] basic assumptions have been made to provide an explanation on several aspects of such a model. More specifically, when the cell is either suspended or at rest, the binding proteins or integrins are rearranged over the interface between the cell and the surface.[10] Furthermore, the short actin filaments inside the cytoplasm are encompassed by actin monomers which are bound to profilin. Myosin II is bent, and the tail domain interacts with the motor head. Moreover, the stress fibers are formed through either a nervous impulse or an external signal.[37] When there is no applied tension, the actin filaments are free of rein by the bipolar myosin filaments, leading to the disassembly of the stress fibers. These mechanisms lead to the conclusion that tensile forces are critical for the formation of stress fibers and that the cells can respond to any restraining forces through a greater tensile load on the integrins.

In addition, the following assumption must be considered.[23] The stress fibers can be uniformly activated and formed in each direction. Hence, the signal of the triggering event can be modeled as an exponential function of time. This triggering affects the activation level of the stress fibers. When there is full activation, then the maximum stress is applied and can be related with the strain rate of the tissue. Utilizing a version of the Hill equation[38] and taking into account the fiber lengthening, we can attain the hyperplastic

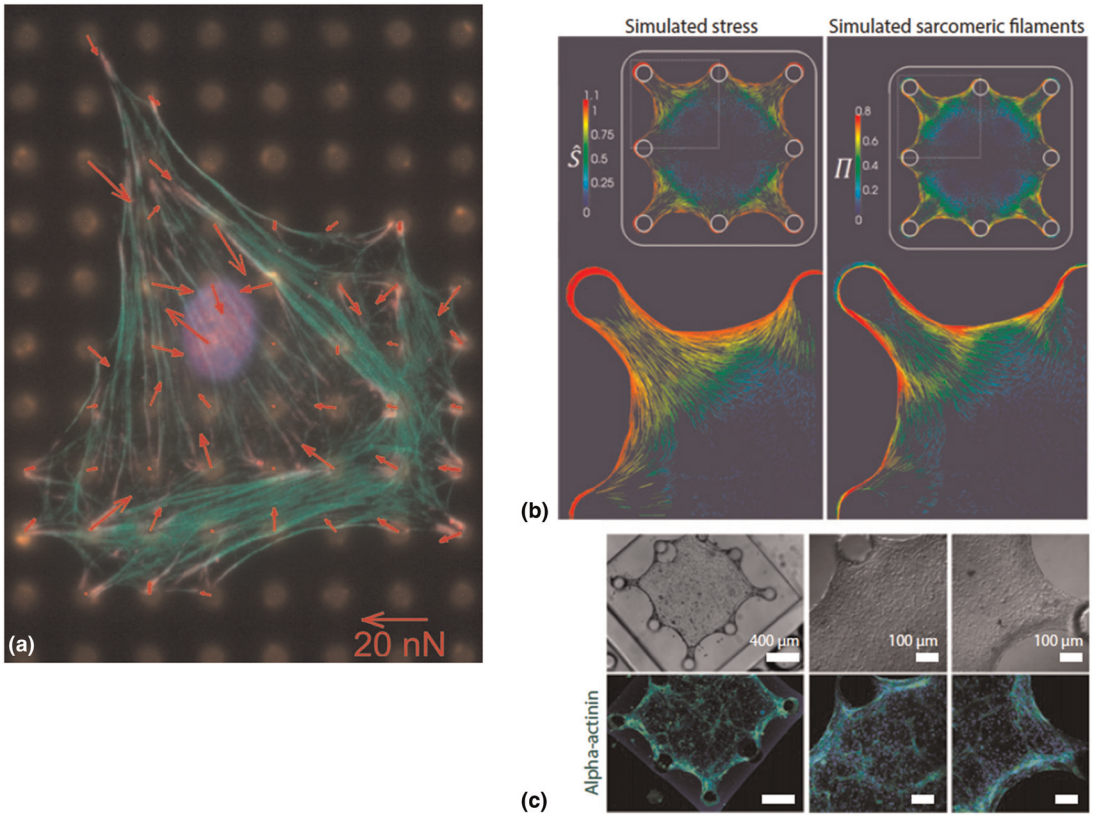

Figure 2. Evaluation of the constitutive modeling in individual cells and tissue. (a) Estimation of the contractile forces in a fibroblast cell, layered on a bed of microneedles. The actin fibers are stained in green. The arrows point the deflection of the posts. The lengths of the arrows are proportional to the magnitude of the force exerted by the cell on the posts.[23] Courtesy of Christopher S, Chen of Boston University. (b) Simulation results of cardiac tissue showing the normalized stress distribution $\hat{S}$ and the expression of sarcomeres Π. (c) The fluorescent imaging of the stained sarcomeric α-actinin (green) confirms the estimated expression by the FEA analysis.[24] Reprinted with permission from PNAS.

constitutive law of the system. Experiments on cells placed on a bed of microneedles and compared with the computational model show that there is a decrease in the forces that the cell generates as the substrate becomes less stiff.[23] In addition, there are strong anisotropic effects that depend on the boundary conditions of the cells and there is high concentration of the stress fibers at the focal adhesions. The characteristic examples of such cells are presented in Fig. 2(a). It must be noted that this model has been employed to tailor the design of aligned and functional 3D cardiac tissues from human pluripotent stem cells.[24] Figure 2(b) shows how heart cells in the ECM can introduce stress alignment and a patterned expression of sarcomeric filaments Π in both simulations and in fluorescent imaging [Fig. 2(c)]. Interestingly, this model correlated regions exhibiting highly aligned sarcomeres with areas of high stresses. Formed sarcomeres are predicted in regions where the local stress state is uniaxial, whereas sarcomeres are not observed in regions where the stresses are biaxial. Hence, uniaxial loading causes highly aligned tissues that express spatially homogeneous contractile proteins. This is a riveting finding since these proteins can be employed for in vitro imitation of cardiac muscle fibers. Nevertheless, it must be noted that this model has been employed for 2D structural systems only, since the full 3D mechanical response is much more challenging to be conveyed from the perspective of both modeling assumptions and numerical evaluation.

However, even without complex models for a facile mechanical prediction, there is significant progress in 3D tissue modeling for in vitro testing. Through MPL, complex microscale structures[14] and arrays[7] can be fabricated such that they can be seeded with cells. More specifically, patterned surfaces are considered to be a powerful arsenal for affecting cellular functions.[39] Cell-trapping well arrays can control the cell shape and behavior. For example, fibroblast cells (NIH-3T3) align more effectively when cells are nested in deeper grooves and narrower ridges.[39] In addition, various cell morphologies can be observed, depending on both the height and the type of the arrays with a threshold of obstacle height equal to 1 μm to permit cell alignment. A characteristic pattern like this is presented in Fig. 3(a). The different cellular morphology depending on the pattern dimensions is presented in Fig. 3(b). Even though this work was still incipient at that point, it elucidated how geometrical parameters of the environment that the cells are positioned can affect them. It must also be remarked that

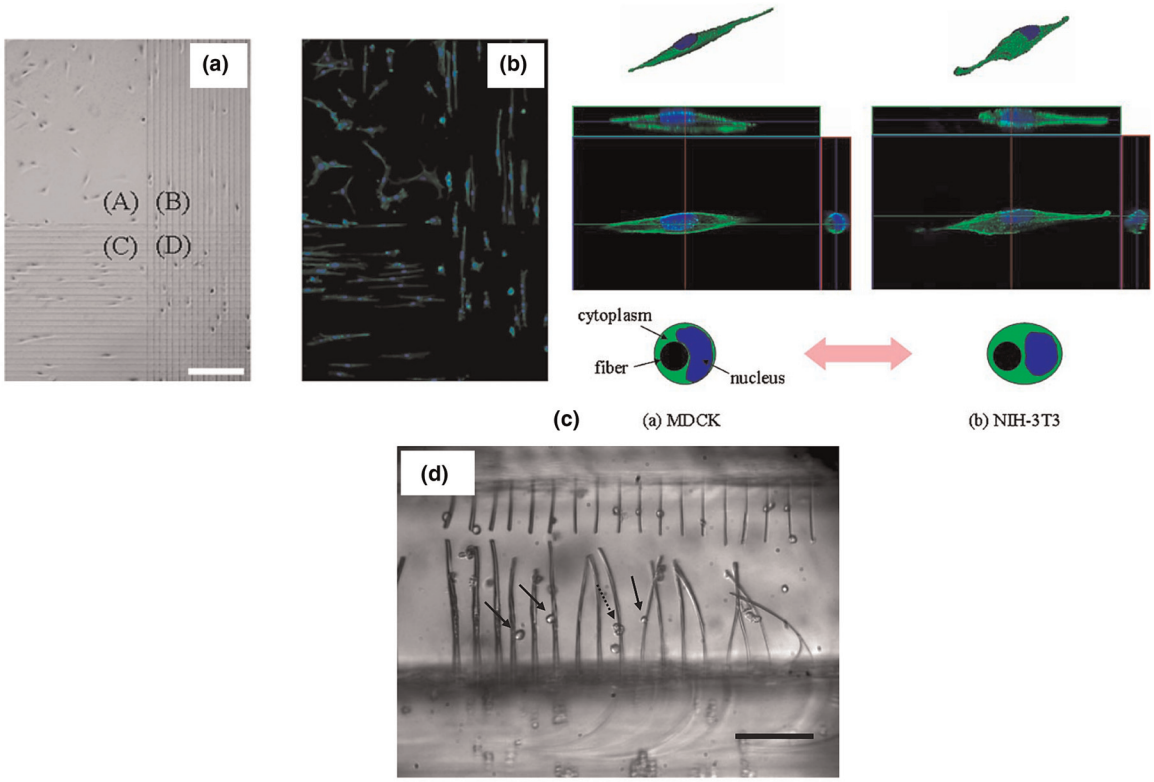

Figure 3. Effect of the patterned surface in the cellular response. (a) Patterned surface observed through scanning electron microscopy. (b) Fluorescent imaging of the cellular alignment on the patterned surface.[33] Reprinted with permission from Wiley. (c) Images of 3D cell morphology, cross-sectional images, and cross-sectional schematics for the individual cells attached on a fiber.[40] Reprinted with permission from Springer. (d) Deformed fibers due to the forces applied by the attached cells.[41] Reprinted with permission from Springer.

the cell morphology is imperative to design biomaterials for tissue engineering. A more realistic observation of the cellular behavior can be reported using complex patterns such as pillars.[40] In particular, a primary objective of such in vitro tissue models is to measure the contractile forces that are generated by the cells.[41] For instance, tractional and contractile forces generated by fibroblastic cells have a significant impact in wound contraction and closure during the healing of an injury. Due to the migration of the fibroblasts, the tractional forces reorganize the cells along stress lines to mechanically precipitate generation of collagen and acquisition of proto-myofibroblast phenotype. Moreover, tractional forces caused by migrating cells can initiate wound contraction. Therefore, it is crucial to measure such forces. An expedient experimental approach is to fabricate cantilever beams through MPL and observe their deformation due to the attachment of cells.[40,41] When a cell has grown on the fiber, an axial compressive force is generated due to the cell contraction. This leads to large deformations on the fiber that can be measured through imaging and correlated with the applied load from the cells. Figure 3(c) demonstrates the imaging of such cells on a fiber. Through this process, it is easy to fathom how the contractile forces that are generated by the cells can affect the deformation of the fibers, leading to a better understanding of the edifice mechanisms of wound healing. Figure 3(d) shows the deformation of the whole fiber due to the loading of the cells. From the perspective of 2D tissue samples as the ones that were reported in the finite element analysis (FEA) modeling, 2D microtissues were attached to columns, again investigating tissue healing properties.[42] These microtissues loaded under tension possess tissue contractions and matrix remodeling, leading to wound closure when the microtissue has a cut. A similar structural paradigm was utilized to investigate the behavior of cardiac microtissues. Nevertheless, the tissue exhibits 3D structural features, leading to effects that a 2D model cannot convey effectively.

To expand this concept one step further, an array of columns can be fabricated to attach a conglomeration of cells on them. Microtissues, with or without the utility of the ECM, are generated through the cessation of cells' merging before they can compact. This occurs when cells adhere to one another and to their proximal ECM. Thus, the density of the microtissue increases. Under this principle, it proved to be enticing to study the behavior of the cardiac tissues in vitro. Human inducing pluripotent stem cells (hiPSCs) and genome-editing tools enable the investigation of physiological phenotypes and the recapitulation of disease pathologies using these the pillar

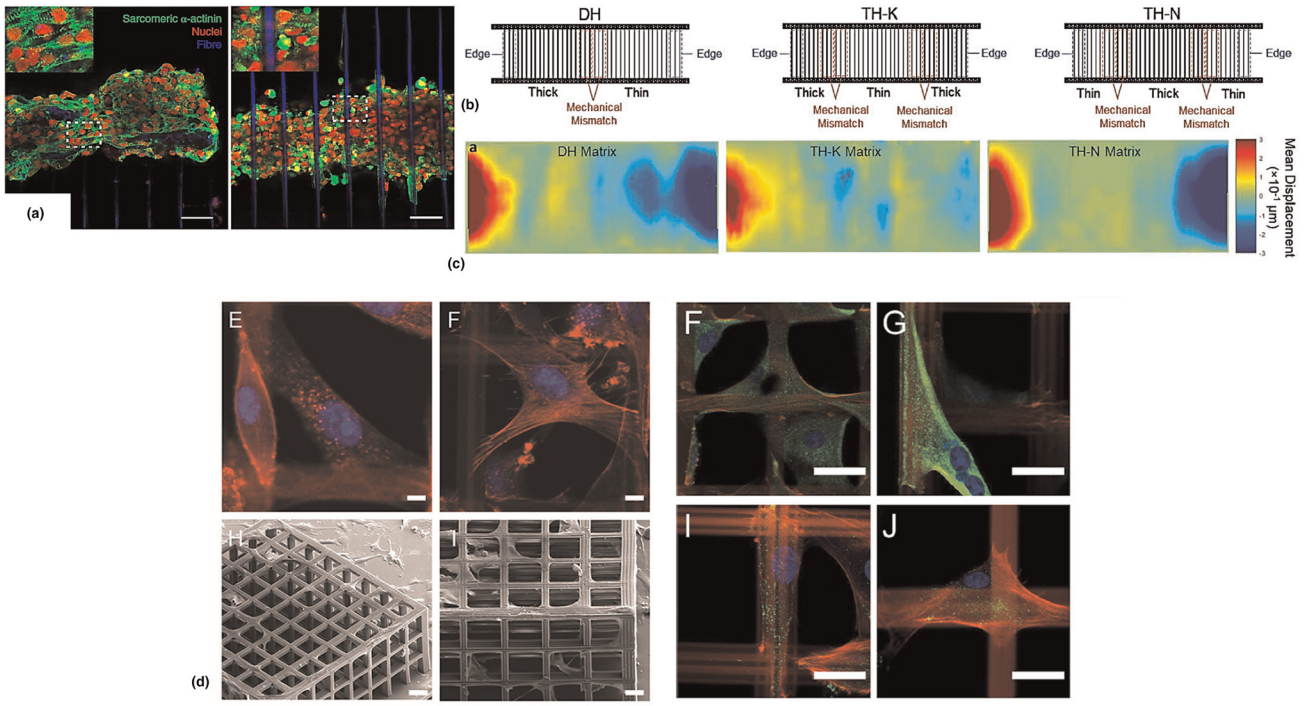

Figure 4. Cellular behavior of cardiac tissue at an either uniform or nonuniform environment. (a) Confocal microscopy images of the MYBPC3$^{-/-}$ cardiac microtissues.[21] Reprinted with permission from Nature. (b) Schematic representation of the nonuniform scaffolds. (c) Displacement heat maps of entire cardiac microtissues for the different nonuniform environments.[26] Reprinted with permission from Wiley. (d) Adhered cells in either 3D (label E) or flat structures (label F), showing significant morphological variation. The SEM images of the 3D structures are shown in labels H and I. The other set of labels (F–J) shows fluorescence images on either flat (F, I) or 3D structures (G, J).[14] Reprinted with permission from ACS Publications.

scaffolds.[21] Moreover, hiPSCs can be utilized to model human heart diseases in the cell culture. Mechanical factors such as the applied stresses exhibit a significantly crucial role in the normal operation of the heart and the pathogenesis of diseases such as cardiomyopathy.[43] Hence, introducing mechanical stresses based on the scaffold morphology into the engineered hiPSC-based tissue models can set the framework to precisely model disease phenotypes. Cardiomyopathies are often related to mutations of myosin-binding protein C cardiac isoform (MYBPC3), a thick-filament accessory protein of the striated muscle sarcomere A-band. To provide an exegesis whether tissue mechanical resistance to contraction can regulate cardiomyocyte sensitivity and disease phenotypes due to the loss of function mutation of MYBPC3, highly ordered 3D fibers have been fabricated and seeded. Due to the mechanical properties of these fibers, cardiac tissue self-assembly and dynamic remodeling can be promoted and observed. Although these fibers have constant mechanical properties, thicker fibers have a higher mechanical resistance to cellular contraction than thinner fibers. Hence, cardiac microtissues produce higher forces when they are developed on the matrices with stiffer fibers. This leads to the manifestation of contractile deficits where the tissue is devoid of the MYBPC3 protein. The characteristic fluorescent images of such tissue models are shown in Fig. 4(a). These results reveal how 3D scaffolds can be employed for in vitro modeling of the external mechanical load to cardiac tissues, either by passive stretch of cardiac tissues mimicking the increase of preload or by stiffening the flexible cantilevers to cardiac tissues mimicking the increase of afterload. These findings can unveil the nuance of abnormalities due to genetic deficiencies in the heart.

Furthermore, nonuniformity of the mechanical properties of the tissue also affects the development of heart dysfunctions. To inquire how this effect manifests itself, 3D cardiac microtissue models with engineered mechanical nonuniformity were also developed. Nonuniformity of the tissue's mechanical environment has been established as one of the essential constituents that regulate cardiac pathophysiology.[44] For instance, it affects the heart's pumping efficiency, inevitably causing heart failure by propagating local cardiac dysfunction spreading through the whole heart. To architect a nonuniform mechanical environment and evaluate the function of hiPSC-based cardiac microtissues, a "pathological" 3D cardiac microtissue model was designed.[26] Different mechanical loads could be accomplished by fabricating the matrices with different fiber thickness. More specifically, fibers with either 5 or 10 μm thickness were in the same designed matrix, in comparison with the uniform thickness of the previously presented case. In addition, the configuration of the nonuniformity was investigated by varying the location of fibers with different

thickness. Figure 4(b) shows the different scaffolds that were tested. Figure 4(c) shows the mean displacement of the tissue for the different designs. Through the characterization of the tissue morphology, contractile motion velocity, motion synchronicity, force generation and kinetics, power output, and energy of contraction, it was revealed that cardiac microtissues possessed a high proclivity to adaptation in a double-hybrid mechanical environment, while adjusting in contraction was limited and exhibited pathological phenotypes in a triple-hybrid mechanical environment. In this particular case, the double-hybrid scaffold possesses only one mismatch boundary (e.g., fibers of different thickness), whereas the triple-hybrid scaffold exhibits two mismatch zones. The configuration of the beam members for these environments is illustrated in Fig. 4(c). These results illuminated how engineering a nonuniform mechanical environment can provide a new avenue to examine in vivo pathological conditions and comprehend cardiac disease progression.

It should be pointed out that the effect of the 3D environment has been investigated for more general biomechanical phenomena, such as cytoskeleton structuration and nucleus deformation. It has been reported that by designing 3D cage-like scaffolds with a variety of topographical features, the effects of induced membrane curvature can be evaluated even at the nanoscale. More specifically, the 3D cage structures have pitch equal to 4.5 μm, and height equal to 500 nm were seeded with U87 cells,[14] as shown in Fig. 4(d). Using confocal and electron microscopy enables the close observation of both cytoskeleton structuration and membrane rearrangement at the material interface. It is reported that cells that adhered on the 3D surfaces have substantially different behavior than those normally found on 2D substrates. On 2D structures, cells tend to match the profile of the surface maximizing the contact with the surface, while the cells attached on the 3D surfaces demonstrated a remarkable membrane deformation encompassing the topographical design. This is a major mechanistic trait since modulation of the shape of mesenchymal stem cells can lead to specific molecular pathways through the rearrangement of the cellular membrane connected to the plasma membrane activity. In addition, cellular functions can be regulated, such as in the tissue homeostasis mechanisms. Despite the fact that this work deviates from results exclusively focused on specific aspects of a healthy tissue, it still demonstrated that the mechanical design of 3D scaffolds can provide compelling findings regarding the cellular behavior.

Architected biological scaffolds

Even though the aforementioned simple structures have provided great insight in the mechanics of the cellular response, in most cases the physical tissue has a much more complex 3D architecture. Therefore, different designs inspired by the extraordinary properties of metamaterials have also been employed to investigate the mechanical performance of the tissue.

The mechanical properties of the architected bioscaffolds can be controlled either by the architecture or their innate material properties. Regarding the mechanical properties, there is a significant progress in the development of heterogeneous micro-mechano-environments through the control of the material. As it was discussed in the previous section, nonuniformity is a major factor for cardiovascular diseases, but also morphogenesis, regeneration, and breast tumorigenesis. A primary technique that can lead to a stiffness gradient in the ECM is oxygen inhibition.[45] Oxygen inhibition can forestall the curing thickness since it obstructs the free radical photopolymerization of the scaffold. Modulating the oxygen inhibition in the layer-by-layer fabrication process modulates the local cross-linking of the polymerized photoresist, consequently controlling the local stiffness of the scaffold. This fabrication technique has been reported to monitor the cellular organization and in vitro tissue reconstruction.[46] It has been demonstrated on oxygen-permeable PDMS. Utilizing atomic force microscopy, it was shown that the materials possess localized stiffness varying between 2 and 15 kPa. Seeding such scaffolds with bovine pulmonary artery smooth muscle cells (bPASMCs) showed a directionality in cellular attachment and morphology. These observations are cogent with the principles that were presented regarding the adherence of cells in either soft or stiff substrates. While this is a major breakthrough in the design of architected bioscaffolds, utilzing architected structural configurations is the primary approach to tune the mechanical properties.

A major category that has been studied is that of zero Poisson ratio scaffolds. When these scaffolds are axially strained, there is no traverse deformation. While this effect has been reported in nature in tendons or skin,[32] it is also expedient to imitate the tissue response during wound healing or tissue ingrowth. Therefore, these scaffolds are auspicious for tissue engineering of the ligament, the cartilage, or the corneal. Zero Poisson ratio can be realized through the design of semi-re-entrant honeycomb structures, which have been thoroughly investigated from the perspective of tissue engineering.[47] More specifically, polyethylene glycol (PEG) materials with the controllable Poisson ratio have been fabricated through projection printing. These materials were selected since PEG hydrogels can be successfully incorporated in 3D cell cultures. It was observed that the cells could successfully get attached to the scaffold, revealing that these designs can be potentially utilized for tissue ingrowth. In addition, seeding these scaffolds with 10T1/2 fibroblast cells and C2C12 myoblast cells showed the aggregation of cell growth. From the mechanical perspective, the dimensions of the structure are critical to obstruct nonspecific autopolymerization since this causes large deformations and ineffective aggregation of the cells. Utilizing a stabilized-rounded' hinge, stiffening of the neighboring regions can be embraced, prohibiting nonspecific autopolymerization. Furthermore, structures with a negative Poisson ratio have been employed to create 3D artificial scaffolds.[48] Utilizing the photoresist SZ2080, 3D bowtie

structures possessing a negative Poisson ratio were fabricated. Structures with a negative Poisson ratio have the same type of deformation in all directions, prohibiting barrel shape formation for the case of compression or necking for the case of tension. Hence, the structure can sustain significantly larger deformations without failure. By performing in situ micromechanical testing incorporated in the scanning electron microscope, it was shown that the unseeded scaffolds can sustain even 60% deformation without fracture. The characteristic deformation regimes are shown in Fig. 5(a). Even though the structure is resilient to fracture, seeding it with fibroblast cells revealed high compliance, as it is presented in Fig. 5(b). This enables the proliferation and migration of cells in the scaffold, rendering it a suitable candidate for tissue engineering. Moreover, the effect of the scaffold's 3D geometry has also been addressed from the perspective of bone tissue growth.[49] Seeding tetrakaidekahedral structures with Osteoblast-like cells (SAOS-2) showed an increase of intracellular f-actin for those structures with higher compliance. This elucidated that osteoblasts are highly sensitive to substrate elasticity at low stiffness. Again, it was proved that the distribution of actin filaments depends on the substrate stiffness, which is consistent with the rest of the reported results. In addition, the activation of f-actin concentration was calculated utilizing linear elasticity, providing a close match between the experimental results and a simple phenomenological model. In addition, the scaffold architecture has also been investigated with respect to its effect on neuronal networks. The brain can be considered as a 3D structure,[50] requiring a complex 3D architected to imitate the mechanical environments of the neurons. By designing hollow tower structures by MPL, a complex network of neurites can be led to predefined pathways in 3D space.[17] This enables the neurites to be rearranged inside the cavities of the scaffold. In addition, the electrophysiology of the tissue was evaluated through patch-clamp measurements. These experiments revealed that the neuronal function of nerve cells is not hampered even in the reclusive environment of hollow architectures.

Although the importance of spatial mechanical heterogeneity in the diseased tissues has been explored by the development of different scaffold structures,[26,51] it is still challenging to enhance the structural complexity to the similar level as that of natural ECM. To resolve this, metamaterials with ultrafine internal structures and flexible cellular geometry could be used for engineering more delicate spatial mechanical variations.[52,53] Metamaterials also make it feasible to broaden the microscopic architectures to mimic distinct mechanical microenvironments within different tissue types, which is the critical step toward establishing tissue-specific disease models to uncover the detailed impact of mechanical signals on cellular pathological responses. In addition, the highly ordered periodic structure of metamaterials makes it possible to investigate the spatial distribution of mechanical force using appropriate computational tools,[54–56] which is not achievable with bulk materials or animal models. Moreover, the mechanical heterogeneity could be escalated to an extreme level to amplify cellular responses to the level of severe pathology, which might be only recognizable within the animal models. In general biomaterial field, it is hard to decouple the effect of microscopic structures and macroscopic stiffness on the cellular signaling, responses, and remodeling.[57,58] However, changing the densities of cellular units in pentamode metamaterials would not affect the effective mechanical properties,[28,59] which potentially offers a new solution to investigate the independent role of ECM structure and rigidity in the disease progression.

Apart from the spatial characteristics of the native cellular environment, the dynamic nature of incessant reorganization of ECM is another significant challenge in the field of biomaterials.[60,61] Therefore, metamaterials with a dynamic conformational change could serve as more advanced cell culture scaffolds in tissue engineering applications. Many dynamic scaffolds made of stimuli-responsive materials have been used for studying the progressive remodeling of various cells and tissues.[62–65] For instance, mesenchymal stem cells (MSCs) showed better osteogenic differentiation on alginate hydrogel with the temporal stress relaxation property.[66] Thermoresponsive shape-memory polymers (SMPs) have been utilized to reveal the cell remodeling stimulated by the substrate topographical transition.[67,68] Compared to these systems, dynamic metamaterials would provide more possibilities of shape-changing modes, programmable shape-changing rate and magnitude. 3D printed SMP-based metastructures have shown multiple shape-changing configurations during the heat-cooling cycles.[69] The architectural modulation shares high similarity with native ECM reorganization, which could be utilized to recapitulate the gradual change of the mechanical environment under pathological conditions. Furthermore, the flexible morphology of this material enables it to become potential carriers for noninvasive large tissue transplantation, which is difficult to achieve with current delivery tools.

All of these complex structures, applied at a plethora of different cells, elucidate the importance of architectural complexity to design bioimplants that would be feasible to living organisms. Nevertheless, more complex design principles have also to be taken into account for the design of implants and will be presented next.

Design of meta-implants

In the previous analysis, regarding the correlation between scaffold topology and cellular mechanical behavior, all of the related phenomena were investigated at the microscale. While this is critical to comprehend how cells behave at different environments and provide an explanation of the constitutive behavior of mechanobiological systems, it is also critical to improve the mechanical performance of constituent parts at a larger scale. Hence, there is an inexorable advance in the design of implants that embrace design principles from mechanical metamaterials[31] (e.g., meta-implants). The principal objective is to improve bone tissue growth, diminish infections, as well as provide enhanced mechanical performance.[70] Properties such as

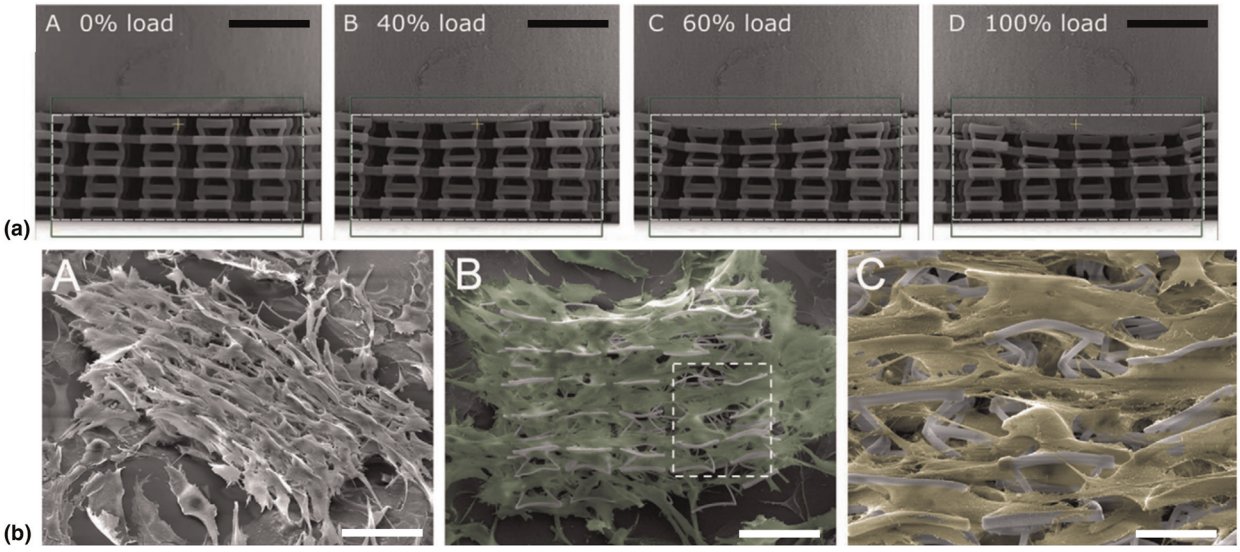

Figure 5. 3D auxetic scaffolds for tissue engineering. (a) Mechanical testing on the unseeded specimens reveals resilience to fracture due to the auxetic behavior. (b) Seeding the scaffolds with cells reveals high compliance, rendering them efficient for tissue engineering.[48] Each length scale bar is equal to 20 μm. Reprinted with permission from Wiley.

negative Poisson's ratio or high stiffness for low weight are also observed in nature.[71] Therefore, properties, which from a conventional perspective would be considered "meta", have evolved in nature through natural selection and must be imitated by the architected design. Nevertheless, even though nature is the paragon of articulated design, there are ways to improve the mechanical performance even more. This is due to the fact that there are states where the performance of the tissue is not the desired one. A characteristic example is tissue regeneration's spatial and temporal limitations. A trademark of an implant must be to match the anatomy of the part that it will replace. Therefore, functionalities such as deployability and shape-morphing have been developed to assist in that endeavor. Deployable meta-implants can be used for minimum surgical invasiveness.[28] Before they are placed in the patient, they possess a state of the minimum surface area, which is reconfigured to obtain its full size and its full loading capacity. This notion is inspired by stents, which change their geometrical configuration when placed inside a blood vessel.[72] However, orthopedic implants need to withstand much larger compressive forces to sustain the body weight compared to the small tensile forces introduced by the blood flow. This is a major design constraint that must be taken into account when designing deployable meta-implants. An expedient way for the design to be deployable is through multistability.[30,31] Multistable mechanisms have more than one stable configurations that they can remain at rest. Each equilibrium point is associated with a specific geometrical configuration and it requires a specific amount of energy to be reached. The characteristic designs are presented in Fig. 6(a). For such designs,

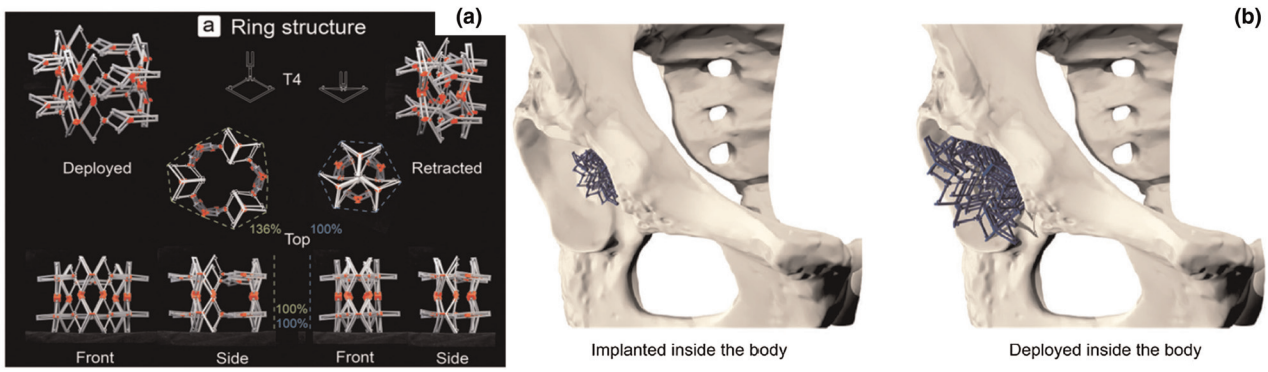

Figure 6. Design and implementation of bioimplants. (a) Snap-through designs for bioimplants. (b) Arrangement of the implant in the human body, at the two distinct equilibrium states.[31] Reprinted with permission from Royal Society of Chemistry.

bistability was accomplished through a snap-through mechanism, which is a form of buckling.[73] The new state that the implant will have with respect to the initial one is shown in Fig. 6(b). Another way to accomplish different equilibrium positions is through origami, kirigami, and multilayered designs. These designs have superior mechanical performance from the perspective of both strength and surface area. Another concept that has been suggested is that of shape-morphing and shape-locking.[74] These implants will possess a shape that's characteristic for the specific patient (i.e., shape-morphing) and then will remain at this equilibrium position (i.e., shape-locking). However, this design required many kinematic mechanisms such that it can alter its shape and then be constrained at a specific position, rendering it significantly more complex than deployable implants. However, bone tissue has multiple different aspects that are essential for it to be functional. The characteristic examples are mass transport or biological properties such as the porosity. Therefore, the architected designs utilized for metal implants must also possess properties from different domains to become truly functional. These properties are achieved by microscale design of the morphology of the implant. While there are not many cases that have been demonstrated experimentally, as it was addressed in the previous section, several concepts are also suggested for potential utility. Structures comprised of beam members or thin-walled structures have been thoroughly investigated regarding multiphysics properties. From the mechanical perspective, they can possess extreme densification when loaded, due to the post-contact of their members during buckling. This ushers enhanced strain energy density and stiffness.[75] Moreover, another category of structures, called triply periodic minimal surfaces,[76] possess elastic modulus similar to that of the bone, very high-yield strength, extremely long fatigue lives, and transport imitating the bone structure. In addition, pentamode structures can be utilized for bone tissue regeneration since their mechanical properties are affected by specific regions of their geometry. This provides the control of their porosity, such that regeneration can be realized. Furthermore, larger-scale structures with a negative Poisson ratio can also be utilized. Polycaprolactone nanofiber membranes have been fabricated and have been proved to possess an almost tenfold elongation capacity. Thus, they can affect the lineage differentiation of the cells.[77] However, such designs have only been tested under mechanical distraction (e.g., a specific loading condition and not a complex mechanical environment). Therefore, this concept has not been proved as a promising solution for tissue growth yet. While all of these designs bear great potential for advanced implants, their commercial utility is still incipient. The reason for this is that conventional implants are competent and clinically safe to be utilized. Thus, future work must also be focused on actual trials of such systems to investigate whether they are indeed efficient. It must also be pointed out that the vast majority of the metamaterial properties have only been addressed in macroscale. Since there is a deep understanding of mechanobiology, incorporation of novel designs such as reconfiguration and bistability in the ECM or scaffold has not been realized. This provides a new avenue to design in vitro microscale tissue models inspired by the concepts of metal implants, which have been thoroughly investigated in the macroscale.

Conclusions

In conclusion, the mechanical modeling of the cellular behavior and the engineering design and scaffolds were summarized. There are many physiological mechanisms associated with the interaction between cells attached to the substrate that affect their mechanical performance. These effects occur in both the linear elastic and nonlinear domain and have been efficiently captured for 2D structural models. In addition, the mechanisms of proliferation, migration, and differentiation can also be associated with mechanical phenomena, providing a deeper understanding in ways to improve the established models. Moreover, the architecture of scaffolds, with properties such as stiffness and geometrical configuration, has significant implications in the normal or malfunctional operation of the cells. While this principle has been efficiently utilized to study cardiac diseases, more complex environments inspired by architected materials will also pave the way to observe intricate functions of the cells in a 3D environment. Perplexed architectures have been efficiently utilized from the perspective of tissue growth and penetration of the scaffold, revealing a potential convergence of the two fields. In addition, larger-scale structures, used as implant for potential surgical operations, namely meta-implants, have demonstrated how the concepts of architected materials can be utilized for the improved behavior of the part of the human body that needs to be replaced. While all of these domains orbit around the concepts of fundamental mechanics, mechanobiology and metamaterial design, they are still not integrated together. However, this fact elucidates that there are plenty of complex environments, embosoming bistability or tailored buckling, that could potentially lead to different patterns in the cellular behavior. Since MPL has been successfully utilized for the design of complex structures, it will be a vital arsenal to achieve this goal. Therefore, combining the additive manufacturing technology of MPL with tissue modeling and architected scaffold design can potentially merge all of these fields to realize complex cellular responses, create bioimplants and study malfunctions of the human body simultaneously. This will elevate the tissue engineering design and advance our understanding of enhanced physical performance.

References

1. L. Li, J. Eyckmans, and C.S. Chen: Designer biomaterials for mechanobiology. *Nat. Mater.* **16**, 1164–1168 (2017).
2. W.J. Polacheck, M.L. Kutys, J.B. Tefft, and C.S. Chen: Microfabricated blood vessels for modelling the vascular transport barrier. *Nat. Protoc.* **14**, 1425–1454 (2019).
3. M.A. Skylar-Scott, M.-C. Liu, Y. Wu, A. Dixit, and M.F. Yanik: Guided homing of cells in multi-photon microfabricated bioscaffolds. *Adv. Healthcare Mater.* **5**, 1233–1243 (2016).

4. H. Jeon, S. Koo, W.M. Reese, P. Loskill, C.P. Grigoropoulos, and K.E. Healy: Directing cell migration and organization via nanocrater-patterned cell-repellent interfaces. *Nat. Mater.* **14**, 918–923 (2015).
5. J. Fu, Y.-K. Wang, M.T. Yang, R.A. Desai, X. Yu, Z. Liu, and C.S. Chen: Mechanical regulation of cell function with geometrically modulated elastomeric substrates. *Nat. Methods* **7**, 733–736 (2010).
6. V. Vogel and M. Sheetz: Local force and geometry sensing regulate cell functions. *Nat. Rev. Mol. Cell Biol.* **7**, 265–275 (2006).
7. F. Santoro, W. Zhao, L.-M. Joubert, L. Duan, J. Schnitker, Y. van de Burgt, H.-Y. Lou, B. Liu, A. Salleo, L. Cui, Y. Cui, and B. Cui: Revealing the cell–material interface with nanometer resolution by focused ion beam/scanning electron microscopy. *ACS Nano* **11**, 8320–8328 (2017).
8. R.L. Satcher Jr and C.F. Dewey Jr: Theoretical estimates of mechanical properties of the endothelial cell cytoskeleton. *Biophys. J.* **71**, 109–118 (1996).
9. M. Iannone, M. Ventre, L. Formisano, L. Casalino, E.J. Patriarca, and P.A. Netti: Nanoengineered surfaces for focal adhesion guidance trigger mesenchymal stem cell self-organization and tenogenesis. *Nano Lett.* **15**, 1517–1525 (2015).
10. C. Storm, J.J. Pastore, F.C. MacKintosh, T.C. Lubensky, and P.A. Janmey: Nonlinear elasticity in biological gels. *Nature* **435**, 191–194 (2005).
11. K. Burridge and M.C. Wodnicka: Focal adhesions, contractility, and signaling. *Annu. Rev. Cell Dev. Biol.* **12**, 463–518 (1996).
12. D.E. Discher, P. Janmey, and Y.-l. Wang: Tissue cells feel and respond to the stiffness of their substrate. *Science* **310**, 1139–1143 (2005).
13. T. Yeung, P.C. Georges, L.A. Flanagan, B. Marg, M. Ortiz, M. Funaki, M. Zanir, W. Ming, V. Weaver, and P.A. Janmey: Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. *Cytoskeleton* **60**, 24–34 (2005).
14. F.A. Pennachio, F. Caliendo, G. Iaccarino, A. Langella, V. Siciliano, and F. Santoro: Three-dimensionally patterned scaffolds modulate the biointerface at the nanoscale. *Nano Lett.* **19**, 5118–5123 (2019).
15. W. Zhao, L. Hanson, H.-Y. Lou, M. Akamatsu, P.D. Chowdary, F. Santoro, J.R. Marks, A. Grassart, D.G. Drubin, Y. Cui, and B. Cui: Nanoscale manipulation of membrane curvature for probing endocytosis in live cells. *Nat. Nanotechnol.* **12**, 750–756 (2017).
16. C.J. Bettinger, R. Langer, and J.T. Borenstein: Engineering substrate topography at the micro- and nanoscale to control cell function. *Angew. Chem.* **48**, 5406–5415 (2009).
17. C. Fendler, C. Denker, J. Harberts, P. Bayat, R. Zierold, G. Loers, M. Munzenberg, and R.H. Blick: Microscaffolds by direct laser writing for neurite guidance leading to tailor-made neuronal networks. *Adv. Biosyst.* **3**, 1800329 (2019).
18. E. Kapyla, D.B. Aydogan, S. Virjula, S. Vanhatupa, S. Miettinen, J. Hyttiten, and M. Kellomaki: Direct laser writing and geometrical analysis of scaffolds with designed pore architecture for three-dimensional cell culturing. *J. Micromech. Microeng.* **22**, 115016 (2012).
19. S. Turunen, T. Joki, M.L. Hitunen, T.O. Ihalainen, S. Narkilahti, and M. Kellomaki: direct laser writing of tubular microtowers for 3D culture of human pluripotent stem cell-derived neuronal cells. *ACS Appl. Mater. Interfaces* **9**, 25717–25730 (2017).
20. D. Iandolo, F.A. Pennacchio, V. Mollo, D. Rossi, D. Dannhauser, B. Cui, R. M. Owens, and F. Santoro: Electron microscopy for 3D scaffolds–cell biointerface characterization. *Adv. Biosyst.* **3**, 180013 (2019).
21. Z. Ma, N. Huebsch, S. Koo, M.A. Mandegar, B. Siemons, S. Boggess, B.R. Conklin, C.P. Grigoropoulos, and K.E. Healy: Contractile deficits in engineered cardiac microtissues as a result of MYBPC3 deficiency and mechanical overload. *Nat. Biomed. Eng.* **2**, 955–967 (2018).
22. A. Pathak, V.S. Deshpande, R.M. McMeeking, and A.G. Evans: The simulation of stress fibre and focal adhesion development in cells on patterned substrates. *J. R. Soc. Interface* **5**, 507–524 (2008).
23. V.S. Deshpande, R.M. McMeeking, and A.G. Evans: A bio-chemo-mechanical model for cell contractility. *PNAS* **103**, 14015–14020 (2006).
24. N. Thavandiran, N. Diubois, A. Mikryukov, S. Masse, B. Beca, C.A. Simmons, V.S. Deshpande, J.P. McGarry, C.S. Chen, K. Nanthakumar, G.M. Keller, M. Radisic, and P.W. Zandstra: Design and formulation of functional pluripotent stem cell-derived cardiac microtissues. *PNAS* **110**, E4698–E4707 (2013).
25. I. Sakellari, E. Kabouraki, D. Gray, V. Purlys, C. Fotakis, A. Pikulin, N. Bityurin, M. Vamvakaki, and M. Farsari: Diffusion-assisted high-resolution direct femtosecond laser writing. *ACS Nano* **6**, 2302–2311 (2012).
26. C. Wang, S. Koo, M. Park, Z. Vangelatos, P. Hoang, B.R. Conklin, C.P. Grigoropoulos, K.E. Healy, and Z. Ma: Maladaptive contractility of 3D human cardiac microtissues to mechanical nonuniformity. *Adv. Healthcare Mater.* **9**, 1901373 (2020).
27. P. Soman, D.Y. Fozdar, J.W. Lee, A. Phadke, S. Varghese, and S. Chen: A three-dimensional polymer scaffolding material exhibiting a zero Poisson's ratio. *Soft Matter* **8**, 4946–4951 (2012).
28. A.A. Zadpoor: Meta-biomaterials. *Biomater. Sci.* **8**, 18–38 (2020).
29. M.E. Yildizdag, C.A. Tran, E. Barchiesi, M. Spagnuolo, F. dell'Isola, and F. Hild: A multi-disciplinary approach for mechanical metamaterial synthesis: a hierarchical modular multiscale cellular structure paradigm. In *State of the Art and Future Trends in Material Modeling*. Advanced Structured Materials, Vol. 100 (Springer, Cham, 2019).
30. F.S.L. Bobbert, S. Janbaz, T. van Manen, Y. Li, and A.A. Zadpoor: Russian doll deployable meta-implants: fusion of kirigami, origami, and multistability. *Mater. Design* **191**, 108624 (2020).
31. F.S.L. Bobbert, S. Janbaz, and A.A. Zadpoor: Towards deployable meta-implants. *J. Mater. Chem. B* **6**, 3449–3455 (2018).
32. P. Mardling, A. Alderson, N. Jordan-Mahy, and C.L.L. Maitre: The use of auxetic materials in tissue engineering. *Biomater. Sci.* **8**, 2074–2083 (2020).
33. H. Jeon, H. Hidai, D.J. Hwang, and C.P. Grigoropoulos: Fabrication of arbitrary polymer patterns for cell study by two-photon polymerization process. *J. Biomed. Mater. Res.* **93A**, 55–66 (2010).
34. J. Swift, I.L. Ivanovska, A. Buxboim, T. Harada, P.C.D.P. Dingal, J. Printer, J.D. Pajerowski, K.R. Splinler, J.W. Shin, M. Tewari, F. Rehfeldt, D.W. Speicher, and D.E. Discher: Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. *Science* **341**, 1240104 (2013).
35. W.J. Polacheck and C.S. Chen: Measuring cell-generated forces: a guide to the available tools. *Nat. Methods* **13**, 415–423 (2016).
36. J. Eyckmans, T. Boudou, X. Yu, and C.S. Chen: A Hitchhiker's guide to mechanobiology. *Dev. Cell* **21**, 35–47 (2011).
37. C. Mohrdieck, A. Wanner, W. Roos, A. Roth, E. Sackmann, J.P. Spatz, and E. Arzt: A theoretical description of elastic pillar substrates in biophysical experiments. *ChemPhysChem* **6**, 1492–1498 (2005).
38. A.V. Hill: The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. B* **126**, 136–195 (1938).
39. H. Jeon, H. Hidai, D.J. Hwang, K.E. Healey, and C.P. Grigoropoulos: The effect of micronscale anisotropic cross patterns on fibroblast migration. *Biomaterials* **31**, 4286–4295 (2010).
40. H. Hidai, H. Jeon, D.J. Hwang, and C.P. Grigoropoulos: Self-standing aligned fiber scaffold fabrication by two photon photopolymerization. *Biomed. Microdevices* **11**, 643–652 (2009).
41. H. Jeon, E. Kim, and C.P. Grigoropoulos: Measurement of contractile forces generated by individual fibroblasts on self-standing fiber scaffolds. *Biomed. Microdevices* **13**, 107–115 (2011).
42. T. Boudou, W.R. Legant, A. Mu, M.A. Borochin, N. Thavandiran, M. Radisic, P.W. Zandstra, J.A. Epstein, K.B. Marguilies, and C.S. Chen: A microfabricated platform to measure and manipulate the mechanics of engineered cardiac microtissues. *Tissue Eng. A* **18**, 910–919 (2011).
43. S.V. Abramson, J.F. Burke, J.J. Kelly, J.G. Kitchen III, M.J. Dougherty, D. F. Yih, F.C. McGeehin III, J.W. Shuck, and T.P. Phiambolis: Pulmonary hypertension predicts mortality and morbidity in patients with dilated cardiomyopathy. *Ann. Intern. Med.* **116**, 888–895 (1992).
44. A.H. Sadeghi, S.R. Shin, J.C. Deddens, G. Fratta, S. Mandla, I.J.K. Yazdi, G. Prakash, S. Antona, D. Demarchi, M.P. Buijsrogge, J.P.G. Sluijter, J. Hjortnaes, and A. Khademhosseini: Engineered 3D cardiac fibrotic tissue to study fibrotic remodeling. *Adv. Healthcare Mater.* **6**, 1601434 (2017).
45. D. Dendukuri, D.C. Pregibon, J. Collins, T.A. Hatton, and P.S. Doyle: Continuous-flow lithography for high-throughput microparticle synthesis. *Nat. Mater.* **5**, 365–369 (2006).
46. H. Yin, Y. Ding, Y. Zhai, W. Tan, and X. Yin: Orthogonal programming of heterogeneous micro-mechano-environments and geometries in three-dimensional bio-stereolithography. *Nat. Commun.* **9**, 4096 (2018).

47. J.J. Warner, A.R. Gillies, H.H. Hwang, H. Zhang, R.L. Lieber, and S. Chen: 3D-printed biomaterials with regional auxetic properties. *J. Mech. Behav. Biomed. Mater.* **76**, 145–152 (2017).
48. G. Flamourakis, I. Spanos, Z. Vangelatos, P. Manganas, L. Papadimitriou, C. Grigoropoulos, A. Ranella, and M. Farsari: Laser-made 3D auxetic metamaterial scaffolds for tissue engineering applications. *Macromol. Mater. Eng.* (2020). doi:10.1002/mame.202000238.
49. A. Maggi, H. Li, and J.R. Greer: Three-dimensional nano-architected scaffolds with tunable stiffness for efficient bone tissue growth. *Acta Biomater.* **63**, 294–305 (2017).
50. G.D. Mahumane, P. Kumar, L.C. du Toit, Y.E. Choonara, and V. Pillay: 3D scaffolds for brain tissue regeneration: architectural challenges. *Biomater. Sci.* **6**, 2812–2837 (2018).
51. H. Ma, A.R. Killaars, F.W. DelRio, C. Yang, and K.S. Anseth: Myofibroblastic activation of valvular interstitial cells is modulated by spatial variations in matrix elasticity and its organization. *Biomaterials* **131**, 131–144 (2017).
52. K. Bertoldi, V. Vitelli, J. Christensen, and M. van Hecke: Flexible mechanical metamaterials. *Nat. Rev. Mater.* **2**, 1–11 (2017).
53. J. Bauer, L.R. Meza, T.A. Schaedler, R. Schwaiger, X. Zheng, and L. Valdevit: Nanolattices: an emerging class of mechanical metamaterials. *Adv. Mater.* **29**, 1701850 (2017).
54. F. Fraternali and A. Amendola: Mechanical modeling of innovative metamaterials alternating pentamode lattices and confinement plates. *J. Mech. Phys. Solids* **99**, 259–271 (2017).
55. J.B. Berger, H.N.G. Wadley, and R.M. McMeeking: Mechanical metamaterials at the theoretical limit of isotropic elastic stiffness. *Nature* **543**, 533–537 (2017).
56. M.J. Mirzaali, A. Caracciolo, H. Pahlavani, S. Janbaz, L. Vergani, and A.A. Zadpoor: Multi-material 3D printed mechanical metamaterials: rational design of elastic properties through spatial distribution of hard and soft phases. *Appl. Phys. Lett.* **113**, 241903 (2018).
57. S. Pok, J.D. Myers, S.V. Madihally, and J.G. Jacot: A multilayered scaffold of a chitosan and gelatin hydrogel supported by a PCL core for cardiac tissue engineering. *Acta Biomater.* **9**, 5630–5642 (2013).
58. K.M. Kennedy, A. Bhaw-Luximon, and D. Jhurry: Cell-matrix mechanical interaction in electrospun polymeric scaffolds for tissue engineering: Implications for scaffold design and performance. *Acta Biomater.* **50**, 41–55 (2017).
59. M. Kadic, T. Bückmann, R. Schittny, P. Gumbsch, and M. Wegener: Pentamode metamaterials with independently tailored bulk modulus and mass density. *Phys. Rev. Appl.* **2**, 054007 (2014).
60. F. Rosso, A. Giordano, M. Barbarisi, and A. Barbarisi: From cell–ECM interactions to tissue engineering. *J. Cell. Physiol.* **199**, 174–180 (2004).
61. K. Uto, J.H. Tsui, C.A. DeForest, and D.-H. Kim: Dynamically tunable cell culture platforms for tissue engineering and mechanobiology. *Prog. Polym. Sci.* **65**, 53–82 (2017).
62. E.A. Corbin, A. Vite, E.G. Peyster, M. Bhoopalam, J. Brandimarto, X. Wang, A.I. Bennett, A.T. Clark, X. Cheng, K.T. Turner, K. Musunuru, and K.B. Margulies: Tunable and reversible substrate stiffness reveals a dynamic mechanosensitivity of cardiomyocytes. *ACS Appl. Mater. Interfaces* **11**, 20603–20614 (2019).
63. Y. Chandorkar, A.C. Nava, S. Scheizerhof, M.V. Dongen, T. Haraszti, J. Köhler, H. Zhang, R. Windoffer, A. Mourran, M. Möller, and L. De Laporte: Cellular responses to beating hydrogels to investigate mechanotransduction. *Nat. Commun.* **10**, 4027 (2019).
64. P.Y. Mengsteab, K. Uto, A.S.T. Smith, S. Frankel, E. Fisher, Z. Nawas, J. Macadangdang, M. Ebara, and D.-H. Kim: Spatiotemporal control of cardiac anisotropy using dynamic nanotopographic cues. *Biomaterials* **86**, 1–10 (2016).
65. C.A. DeForest and D.A. Tirrell: A photoreversible protein-patterning approach for guiding stem cell fate in three-dimensional gels. *Nat. Mater.* **14**, 523–531 (2015).
66. O. Chaudhuri, L. Gu, D. Klumpers, M. Darnell, S.A. Bencherif, J.C. Weaver, N. Huebsch, H.-p. Lee, E. Lippens, G.N. Duda, and D.J. Mooney: Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nat. Mater.* **15**, 326–334 (2016).
67. L.-F. Tseng, P.T. Mather, and J.H. Henderson: Shape-memory-actuated change in scaffold fiber alignment directs stem cell morphology. *Acta Biomater.* **9**, 8790–8801 (2013).
68. S. Sun, H. Shi, S. Moore, C. Wang, A. Ash-Shakoor, P.T. Mather, J.H. Henderson, and Z. Ma: Progressive myofibril reorganization of human cardiomyocytes on a dynamic nanotopographic substrate. *ACS Appl. Mater. Interfaces* **12**, 21450–21462 (2020).
69. C. Yang, M. Boorugu, A. Dopp, J. Ren, R. Martin, D. Han, W. Choi, and H. Lee: 4D printing reconfigurable, deployable and mechanically tunable metamaterials. *Mater. Horiz.* **6**, 1244–1250 (2019).
70. J. van der Stok, H. Wang, S.A. Yavari, M. Siebelt, M. Sandker, J.H. Waarsing, J.A.N. Verhaar, H. Jahr, A.A. Zadpoor, S.C.G. Leeuwenburgh, and H. Weinans: Enhanced bone regeneration of cortical segmental bone defects using porous titanium scaffolds incorporated with colloidal gelatin gels for time and dose-controlled delivery of dual growth factors. *Tissue Eng. A* **19**, 2605–2617 (2013).
71. F. Barthelata, H. Tang, P.D. Zavattieri, C.-M. Li, and H.D. Espinosa: On the mechanics of mother-of-pearl: a key feature in the material hierarchical structure. *J. Mech. Phys. Solids* **55**, 306–337 (2007).
72. R.L. Noah, C. Hanratty, and S.J. Walsh: Clinical impact of stent design. *Interv. Cardiol. Rev.* **9**, 89–93 (2014).
73. Z. Vangelatos, G.X. Gu, and C.P. Grigoropoulos: Architected metamaterials with tailored 3D buckling mechanisms at the microscale. *Extreme Mech. Lett.* **33**, 100580 (2019).
74. S. Leeflang, S. Janbaz, and A.A. Zadpoor: Metallic clay. *Addit. Manuf.* **28**, 528–535 (2019).
75. Z. Vangelatos, V. Melissinaki, M. Farsari, K. Komvopoulos, and C. Grigoropoulos: Intertwined microlattices greatly enhance the performance of mechanical metamaterials. *Math. Mech. Solids* **24**, 2636–2648 (2019).
76. O. Al-Ketan and R.K.A. Al-Rub: Multifunctional mechanical metamaterials based on triply periodic minimal surface lattices. *Adv. Eng. Mater.* **21**, 1900524 (2019).
77. S.K. Bhullar, D. Rana, H. Lekesiz, A.C. Bedeloglu, J. Ko, Y. Cho, Z. Ayatac, T. Uyar, M. Jun, and M. Ramalingam: Design and fabrication of auxetic PCL nanofiber membranes for biomedical applications. *Mater. Sci. Eng. C* **81**, 334–340 (2017).