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Using biophysical cues and biomaterials to improve genetic models

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Abstract

With the advent of induced pluripotent stem cells and modern differentiation protocols, many advances in our understanding of disease have been made possible by *in vitro* disease modeling; in some cases, their use may have supplanted animal models. Yet *in vitro* models often rely on rigid cell culture substrates that could limit our ability to completely reproduce human disease in a dish. Nascent work, however, suggests that the combination of biomaterials and/or advanced microphysiological systems–which better recapitulate tissue properties–with stem cells expressing disease mimicking genetics, could substantially improve current disease modeling efforts where genetics alone is insufficient. This review will highlight such recent advances as well as review current challenges that the fields must overcome to create more personalized therapeutics in the future.

Keywords

Stem cells; Extracellular matrix; Genome editing; Disease modeling; Organoids

Introduction

For decades, animal models have been the gold standard for studying disease pathology, progression, and therapeutics. However, it has become increasingly clear that animal models are simply inadequate for studying an array of pathologies and genetic disorders. Mice, and other animals, lack genetic regions of certain diseases, making them impossible to model [1,2]. And variation in tissue architecture and cellular makeup across species can lead to a mismatch in disease progression, e.g., in fibrosis and scarring [3]. To address these concerns, biomedical research has shifted from animal models to complex *in vitro* models comprised of human pluripotent stem cells (hPSC) because they have the ability to generate patient specific cells and responses [4,5]. When paired with genome manipulation tools, such as

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Declaration of competing interest

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CRIPSR/CAS9, researchers have developed of an assortment of cells that contain genetic profiles of a wide array of pathologies [6], enabling exploration into previously inaccessible diseases. These cell models have been instrumental in providing us with insights into therapeutics and diagnostics for a variety of disorders. However, when used on plastic dishes without the biophysical properties of the tissues they aim to emulate, they fail to capture the totality of human pathologies.

Concurrently, excellent work has been done developing a suite of advanced microphysiological systems that can recapitulate complex tissue architectures, native cellular heterogeneity and organization, and tissue matrix properties and dynamics [7,8]. However, the application of these platforms in conjunction with the genetically defined cell disease platforms to interrogate genetic disorders has remained underexplored. The aim of this review is to highlight recent advances in both engineered systems (Figure 1) and patient genetic engineering studies for disease modeling. We finish with discussing current challenges in linking these fields together, which could establish the next frontier in personalized medicine and disease modeling.

Microphysiological systems for tissue modeling

Microphysiological systems (MPS) are *in vitro* models that aim to emulate native tissues using engineering principles. Initial MPSs were primarily microfluidic organ-on-a-chip systems; however, recent advances in 3D bioprinting and embedded organoid materials systems have expanded the MPS domain (Table 1) [8]. These systems provide a few advantages over standard tissue culture as they often have perfusive flow paired with biophysical and chemical cues, emulating complex organoid functions as well as inter- and intra-organ interactions. Most systems have spatial control over cells and matrices, with some examples providing highly accurate representations of native tissue architectures [9,10]. Endowed with these advantages, they have been used to study embryogenesis and tissue development, disease progression, and drug efficacy for preclinical screen and toxicology [11].

One overarching feature across various microphysiological systems is compliant matrices. These matrices provide native cells with physiologically relevant cell attachment ligands, porosities, and mechanical properties such as stiffness and stress relaxation. And these properties can alter cell phenotypes through mechanotransduction pathways [12]. Alterations in those pathways, via biophysical cues from complaint matrices, play a clear role in guiding cell functions such as stem cell differentiation and cell migration [13,14]. While having cells on or in a compliant matrix alone is not sufficient to be classified as a microphysiological system, their inclusion is critical when developing engineered tissue models.

The first MPSs developed were microfluidic devices [15–17]. Typically, devices were fabricated with PDMS using soft lithography to create chambers for cells and hydrogels, as well as inlets and outlets that allow for fluid flow. By modifying the geometry and location of inlets, outlets, and chambers, cells can be exposed to direct flow, interstitial flow, and gradients. This allows for easy fabrication, optical clarity for imaging, and

high reproducibility. Extensive work over the years have established robust systems for lung, kidney, liver, gut, bone, vasculature, endometrial tissue, and more [7,18–20]. Recent work has also expanded on organoid-on-a-chip systems by directly embedding or generating whole organoids in conjunction with materials systems. For example, Gjorevski et al. explored how tissue patterning could direct intestinal organoid morphology using a photosensitive hydrogel matrix to temporally pattern geometrically defined cavities [21]. Other work has explored embedded cerebral organoids with an air liquid interface [22], and some studies have begun using micropatterning to geometrically confine cells to form cardiac, liver, cerebral, and intestinal organoids [23,24].

The advent of bioprinting has also provided exciting opportunities for tissue models as it allows for the integration of multiple cell types and varied matrices with high spatial control. Typical bioprinting fabrication techniques, such as extrusion and inkjet printing, use layer by layer deposition of inks to build up a tissue construct. Notable recent examples include printing of thick vascularized intestines [25] and the high throughput generation of centimeter scale patches of kidney organoids with a dense patterning of nephrons and functional proximal tubules [26]. Recently, embedded 3D printing has gained traction as it allows for freeform printing of structures, enabling the construction of more complicated tissue architectures [27,28]. This has been applied to the formation of functional heart valves to study heart valve disease [10], as well as the creation of freeform vasculature in centimeter sized cubes of cardiac and cerebral organoids [29].

While these systems have been indispensable for their ability to mimic many properties of their physiological counterparts, there remains significant scope for combing these tools with genetically engineered cell systems.

Patient genetic disorder models

The generation of patient models of genetic disorders has been instrumental in advancing preclinical research. The first models were generated using primary patient cells isolated directly from tissue [30]. These provide a clear advantage over both animal models and established cell lines as primary cells contain the exact genetic makeup of real risk and non-risk populations. Then, the ability to generate hPSCs from patients afforded researchers further control as they are a perpetually renewing cell source, limiting the amount and frequency of tissue acquisition as well as the type of tissue needed. In addition, there is now the ability to knock in mutations into healthy patient cell lines, or knock out mutations in risk patient lines. The genetic abnormalities in any of those cell models can range from single base pair mutations to complex multifaceted genetic changes that may still be unknown, such as with Alzheimer’s [31]. The more complex the genetic makeup of a disorder, the more difficult it is to recreate that disease’s phenotype in donor lines, emphasizing the utility in establishing large biobanks.

A substantial amount of patient models were performed on tissue culture plastic systems in 2D, typically with a homogeneous cell population. Now, models are quickly moving towards organoids, which are 3-D structures containing complex mixtures of heterogeneous cell populations that give rise to organ-like function. Organoids can be derived from isolated

adult stem cells from patient tissues, or from hPSCs. An array of patient models across most tissues has been developed [32], ranging from single base pair disorders like cystic fibrosis [33,34] to multipoint mutations disorders like cerebral MAPT mutations [35] and congenital nephrotic syndrome [36], to full gene knockdowns including polycystic kidney disease [37]. While these advances are substantial, it is imperative that we continually expand our platforms to provide scalable, simple, and accurate models of pathologies relating to tissue structure with biologically relevant temporal time scales. Certain diseases are also more complex than just missing cell phenotypes, and they can have complex modes of progression. For example, diseases that alter matrix composition with progression states tied to the mechanics of the surrounding matrix cannot be modeled merely through suspension organoids or static 2D tissue culture plastic models alone [38]. In this next section we will highlight the few key examples of MPSs combined with genetic disease cell models, and we will discuss which gaps we've started to fill and where the future challenges in this space reside.

Future genetic modeling: combining engineered systems and genetics

There is a spectrum in complexity for genetic diseases, from identifiable phenotype changes due to only single base pair mutations, to complex hereditary disorders with adult-onset symptoms and unknown drivers of progression [31,39]. Patient models have been instrumental in providing fundamental understandings into how many "simple" genetic diseases develop and progress, giving new targets for therapeutics. However, the further we slide along the complexity spectrum, the more we need complements to pair with genetic engineering tools to create adequate models. Pairing genetic patient models with MPSs is a clear and direct way to accomplish this goal.

A few studies utilizing this combination have centered on disorders related to tissue architecture, matrix development, and cellular organization (Figure 2). For example, Achberger et al. created a retinal organoid-on-a-chip to model intravitreal delivery of adenovirus vectors to screen patient gene therapy transduction efficiencies [40]. Their device gave them high control over the structural parameters of the organoids, and the perfusive flow enabled a reproducible delivery that spherical retinal organoids cannot produce. Some works have created 3D heart tissues of contracting cardiac cells to understand contraction dysfunction at a tissue level [41,42]. One group uncovered a novel MYH7 mutation that modeled adult-onset systolic cardiomyopathy [41], while another used CRIPSR/CAS9 on Duchenne muscular dystrophy patient cells to repair contractile phenotypes [43]. And recently, Abudupataer et al. modeled bicuspid aortic valve disorders in microfluidic pressure chamber chips to measure tissue contractions for healthy versus disease patient donors [42].

Another major driver for combining patient models with microphysiological systems is that some disease phenotypes will not appear unless under physiologically relevant conditions. For example, some cell phenotypes appear only under the cyclical loading, shear, and/or tissue extension conditions found in lungs, joints, stomach, the heart, and more. Other disease phenotypes become present during wound repair due to dynamically changing mechanics and matrix compositions. We recently demonstrated a disease phenotype associated with the long noncoding RNA ANRIL that only emerges when cardiac cells

are placed on dynamically stiffened matrices [44]. Other work has explored the use of microfluidic chips to create model airways of cystic fibrosis patients to studying bacterial infections. Here, they showed greater growth of bacteria for cystic fibrosis patient cells only in the chip model, with no difference between the control and patient models on tissue culture plastic [45]. Together, these highlight that certain diseases require context appropriate microenvironments to be accurately mimic the *in vivo* disease pathology.

Many diseases are also multifactorial in their cellular makeup/affection, making them difficult to model with conventional methods. Giacomelli et al. demonstrated this by creating heart tissues comprised of cardiomyocytes, endothelial cells, and cardiac fibroblasts. By using cardiac fibroblasts from patient lines with a PKP2 mutation, they showed that these fibroblasts could induce misfunction of the healthy cardiomyocytes and produce a model of arrhythmogenic cardiomyopathy [46]. By combining mutated patient hPSC derived endothelial cells in a fibrin hydrogel-on-a-chip, Orlova et al. replicated the phenotype of Hereditary hemorrhagic telangiectasia for the first time [47]. Other examples include a gut-on-a-chip model to study patient specific irritable bowel disease with macrophages in the lumen of the structures to look at immunoregulatory crosstalk via cytokine signaling [48], and microfluidic blood brain barrier models with MCT8-deficient T3 transport cells using real blood to predict patient specific drug permeability [49].

These state-of-the-art studies represent the first steps exploring the importance of establishing physiological contexts to genetic patient models. However, these results beg the question: if using microphysiological systems for genetic disorders gives a significant advantage over regular tissue culture plastic and suspension organoid culture, why are they rarely used? First and foremost, there is a high level of expertise required to create both MPSs as well as genetically engineered patient derived models, and it is challenging to establish both within a single lab. Specific difficulties include the need to hire a large yet diverse team with a broad skillset across biology, engineering, and material science with access to lab infrastructure and equipment relevant to all three disciplines. This can be quite expensive, making cost the general limiting factor. Going forward, two effective solutions to provide easier accessibility include: (1) having companies sell cheap, customized microfluidic chips; and (2), having core facilities that provide biobanks of pre and post genetically modified patient cell lines.

We also lack adequate materials that are reproducible, well defined, and support the tissue function and growth while simultaneously being have tunable biochemical and biophysical properties as every disease state is associated with a unique matrix composition and property set. In addition, current microphysiological systems still fail to replicate certain tissue architectures that are related to common developmental and adult-onset pathologies, including complex joints, some valves, lymphatics, heart looping and heart chambers, and more. They also lack proper vasculature. While certain lab-on-a-chip models have addressed this issue, models that have larger and more complex architectures, like embedded organoids or bioprinted constructs, still lack proper patent and hierarchical vessel networks. And there is a sever lack of innervation in both organoid cultures as well as many microphysiological systems, limiting our ability to study their influence.

There is another important question that we need to ask: how simple is complex? [50] It is critical that we do not attempt to replicate every detail of a tissue simultaneously. While that goal is admirable for regenerative medicine, researchers developing disease models should aim for enough complexity to provide true insights into key biological questions asked, while being simple enough to provide scalable and reproducible models in a high throughput fashion at a relatively low cost. And sometimes MPSs are completely unnecessary. For instance, well defined and mature organoids in suspension can still provide vital insights into tissue structure function relationships and how attenuation of their genetic landscape may alter and impair this relationship. In addition, if we want microphysiological tools adopted by labs with infrastructure for genomic biology, we need tools to be readily obtainable and simple enough to be operated without extensive expertise. Nevertheless, it is vital that we reach hands across the aisle to foster more collaborations between biologists and engineers.

Conclusions

In this review, we briefly underlined the current space of patient genetic disease models and MPSs, and we discussed the importance in shifting our focus to combining these two tools together. It is important to note that not every genetic disease model should be a complex microphysiological system, but context is important and needs to be taken into consideration when designing new models. Overall, a goal of our field should be to probe more complex disease states through the combination of genetic engineering with tissue and bioengineering approaches. Even as genetic engineering tools and microfabrication techniques each become cheaper, simpler, and more accessible, a concerted effort must be made to build collaborations and explore new techniques so that we can widen our berth of knowledge across more diseases and, ultimately, improve patient outcomes.

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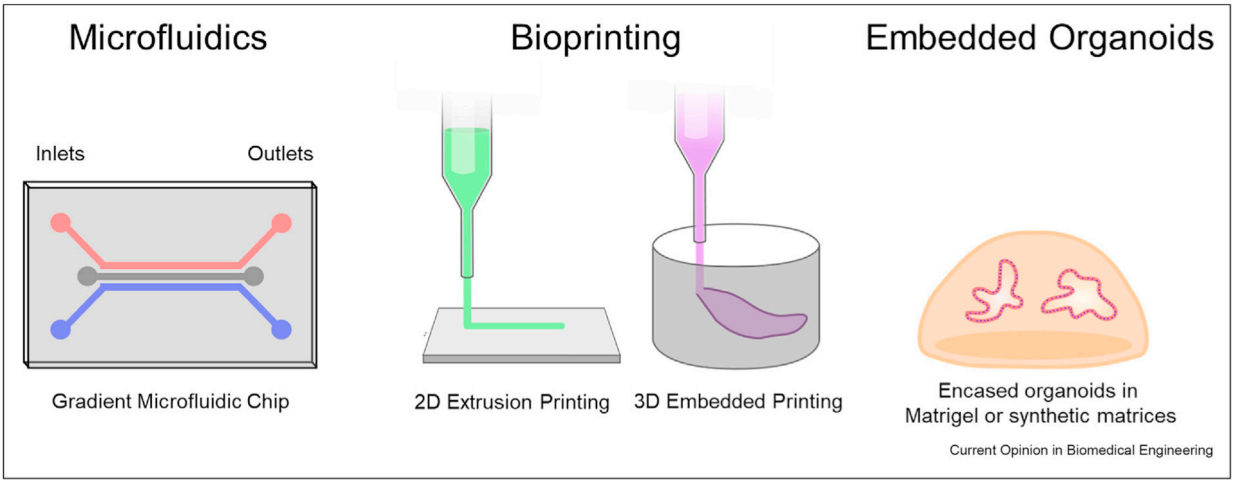

Figure 1.

Types of microphysiological systems. Schematical representation of three common types of microphysiological systems: microfluidic chips for organ-on-a-chip devices (left), bioprinting cellular bioinks (middle), and organoids embedded in hydrogel systems (right).

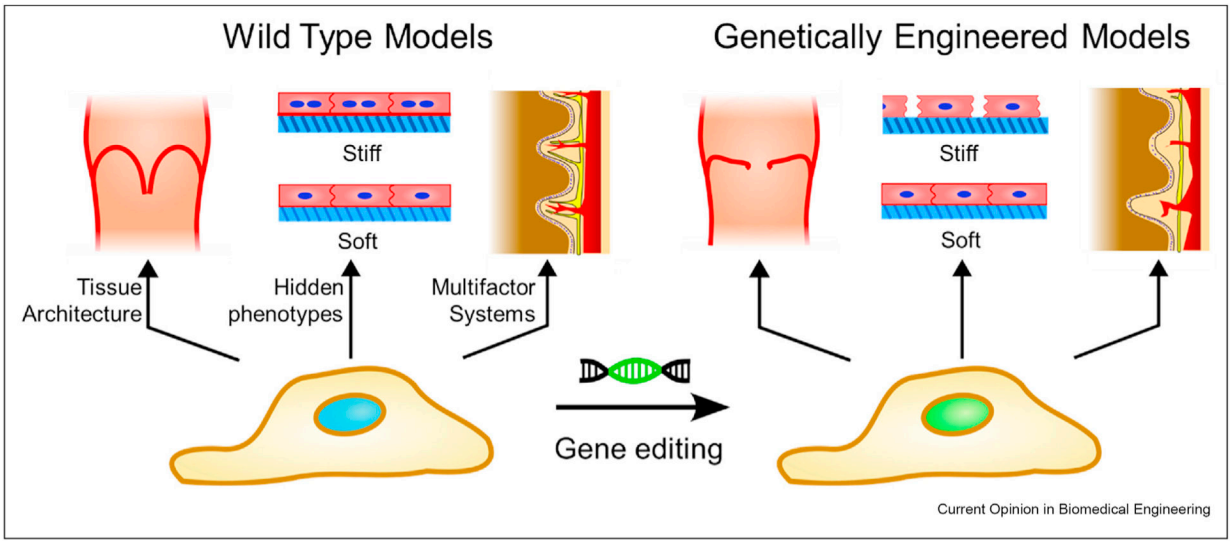

Figure 2.

Genetic engineering paired with MPSs enable the study of tissue scale disease phenotypes. Schematic of the various types of disease studies enabled from combing genetic engineering and MPSs including disruption to tissue architecture, the appearance of hidden cell phenotypes, and the interactions of cells across different systems.

Table 1

Advantages and disadvantages of various microphysiological systems.

	Microfluidics	Bioprinting	Embedded organoids
Advantages	• High reproducibility • High optical clarity • Moderate spatial control	• Highest spatial control • Moderate reproducibility • Can generate complex macro-tissue architectures	• Ease of use • Highest biological complexity • Long term culture
Disadvantages	• Nonstandard culture protocols • Low sample volumes • Requires specialized facilities to fabricate	• Moderate material choice • Requires expensive equipment. • Lower resolution	• Limited material choice • Lowest reproducibility • Less spatial control