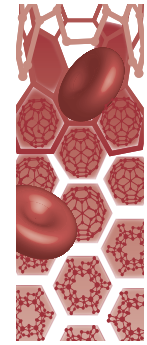
Editorial

Nanomedicine



Seeking the right context for evaluating nanomedicine: from tissue models in petri dishes to microfluidic organs-on-a-chip

"...'human-on-a-chip' systems have potential to function as a robust platform that both applies to fundamental studies of biological agents and diseases, as well as to predicting the effects of nanomedicine in humans."

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Engineering predictive tissue models

The field of tissue engineering and regenerative medicine has seen tremendous progress over the past few decades through a wide spectrum of engineering innovations in biomaterials, biomolecule delivery, biomechanics, biophysics and biomedicine. For example, scaffolds are fabricated to possess controllable structures, porosities, hierarchies, degradability along with well-controlled spatial and temporal presentation of bioactive molecules (e.g., growth factors, antagonists, DNAs and micro/siRNAs) that aid in regulating cellular behavior [1]. Moreover, it is increasingly appreciated that biomechanical cues of the materials can be employed to direct the differentiation of stem cells into specific lineages [2]. Alternatively, cellular behaviors may also be tuned by other biophysical cues including surface roughness and topography [3].

However, since its conception, tissue engineering has always focused on the generation of tissue substitutes to replace those damaged or diseased in the body. Only recently has the area started to enter an emerging paradigm of building physiologically relevant miniature human tissue and organ models. The increasing awareness in animal welfare has further expedited such efforts in generating human tissue models that may eventually replace animal models from the ethical perspective as well as to provide more accurate predictions of human body responses.

Indeed, there have been tremendous progress on developing functional human healthy/diseased organoids from various human cell sources including induced pluripotent stem cells (iPSCs), genetically modified cell lines and diseased cells derived from patients. For example, Helmrath and colleagues showed that through iPSC differentiation and subsequent maturation by transplanting under the kidney capsules of immunocompromised mice, human small intestinal organoids could form that contain mature intestinal epithelium with crypt-villus architecture and a laminated mesenchyme [4]. Also, Knoblich and colleagues demonstrated the potential to generate human brain regions that recapitulated the structure and development of cerebral cortex in 3D iPSC-derived cerebral organoids [5]. In another example, Kim and colleagues created a model of familial Alzheimer's disease (FAD) using a 3D culture system of differentiated neuronal cells expressing FAD mutations, which expressed amyloid-β and phosphorylated tau proteins, similar to those of FAD in human brains [6]. While these examples are still preliminary, the findings have undoubtedly provided significant excitement about generating predictive human tissue models for drug testing. More interestingly, realistic human tumor

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models have also been engineered that represent the tumor characteristics *in vivo*. In a pioneering example, Mooney and colleagues created a 3D model of human oral cancer by culturing oral squamous cell carcinoma cells within highly porous scaffolds prepared from the synthetic biomaterial poly(lactide-co-glycolide), which showed similar levels of biomarker secretion with *in vivo* tumor models, significantly higher than those observed in 2D monolayer cultures [7].

"In order to run a platform with multiple organ types, a common medium, typically referred to as blood surrogate, must be developed to maintain the viability and functionality of all the organs."

A critical limitation in fabricating functional tissue models lies in the inability to drive the cells under *in vitro* cultures to form hierarchical, ordered structures that recapitulate those found in human body. Although it is demonstrated that via rational design of the matrices certain cell populations can sort themselves into simplified biomimetic structures due to the differential mechanics and membrane properties of the cells [8], such capability is rather limited. To address this challenge, advancements have been made in various biofabrication techniques such as 3D bioprinting. Bioprinting holds great potential to surpass the obstacles associated with generating biomimetic tissue architecture by controlling the spatial fabrication of biological architectures, including both cells and extracellular matrix molecules, in a scalable manner [9]. We envision that, with further development of the 3D bioprinting technology, it will be possible to produce tissue and organ models that mimic many aspects of their human counterparts. As an example, biomimetic blood vessels with interconnected lumen structures and a tight layer of endothelium have been fabricated via a sacrificial approach following digitized bioprinting of template materials [10,11]. Besides hollow vascular structures created by sacrificial printing, other tissue-like structures such as skin, bone, airway, heart and cartilage have also been directly bioprinted [9]. It is not unreasonable to assume that these advanced bioprinting techniques can be readily applied to the creation of complex tumor tissues possessing the right cellular/matrix structures and architecture.

Connecting them together: building the human-on-a-chip platform

In human body tissues and organs of all types are interconnected by a sophisticated network of blood vessels. The vascular network enables the communication among different organs, via the transport of biochemical cues and circulating cells. This circulatory system is critically important in allowing for self-containment of the human body by coordinating the functions of different organs at distance. In such an integrated environment neither organs nor their responses are isolated, meaning that the behavior of one organ upon treatment of a substance (e.g., drug) will usually trigger a cascade of reactions of other organs that otherwise do not respond to the molecule by themselves alone. Therefore, in building realistic *in vitro* tissue models it is significant to implement a microfluidic platform where multiple bioreactors housing different organ models are introduced into the same context where the cross-talk among these organs take place, the so-called 'organs-on-a-chip' systems [12–20]. Such models can be made across a range of complexity from either individual or multiple organ types. For example, Takayama and colleagues generated microfluidic airway systems that could be used for studying cellular-level lung injuries [21]. Also, Ingber and colleagues have generated several organ models on chips including lung, kidney, blood vessel, airway and bone marrow [22]. In addition, Shuler and colleagues piloted the micro cell culture analog devices where up to ten organs including tumor units are integrated in order to study their interactions [23]. Similarly, Wikswo and colleagues proposed the microphysiological systems with built-in pneumatic valves to control the 'blood flow' among different organs [24]. Many other collaborative efforts around the world including our own group are at the same time pioneering advanced integration technologies that can advance these biomimetic modules toward the final aim of constructing a viable 'human-on-a-chip' platform.

In order to run a platform with multiple organ types, a common medium, typically referred to as blood surrogate, must be developed to maintain the viability and functionality of all the organs. The scaling effect, defined as the compositional, architectural and functional outcomes of change in size among similarly organized animals, is another major consideration in engineering the organs-on-a-chip platforms. In fact with the variation in organ/body size of vertebrate animal species spanning across a huge range on the magnitude of 10^6, the scaling of parameters such as organ weight, metabolism and blood volume/flow obey different laws. It has been suggested that appropriate scaling laws should be adopted for specific applications depending on whether it is to engineer physiologically functional multiple-organ systems (e.g., beating heart-on-a-chip), pharmacological models of organ interactions, or both [15]. However, we believe that a universal scaling effect may be derived to eventually miniaturize the human physiology onto microfluidic chips that fit for different applications.

Better models for evaluating nanomedicine

The field of nanomedicine has made critical advancements to enable the fabrication of various nanomaterials with different properties that can be used as vehicles for sustained, stimuli-responsive, as well as targeted delivery of therapeutic drugs and diagnostic agents [25]. Tremendous efforts in studying the efficiency and efficacy of nanomedicine have largely relied on 2D cell culture models, but in most cases the results obtained from these studies do not readily translate to *in vivo* scenarios due to the fundamental difference of these over-simplified models comparing with native tissues and organs. For example, cellular uptake and cytotoxicity of nanoparticles in 2D setups are far more efficient and higher than when they are administrated to 3D models that recapitulate the density of native tissues in human body to a much stronger degree than 2D monolayers. Using these 3D tissue/tumor models, the delivery of nanoparticles with different parameters such as size, shape, surface charge and configurations have been investigated [26]. Although improved, these dense 3D constructs may only provide insights on diffusion-mediated drug delivery resembling the stage where the nanoparticles have exited from the blood vessels to reach the interstitial space of the tissues. Therefore, the need to introduce a physiologically relevant microfluidic vasculature into the tumor model seems critical in studying the systemic effects of nanomedicine, which has triggered boosted interest in engineering tumor-on-a-chip platforms [27,28]. Further combination with healthy organs in the circulation not only provides the capability to assess the therapeutic effects of nanomedicine toward tumors but also reveals its systemic side effects on other healthy organs reachable by the blood flow.

Conclusion

There is a strong demand in engineering *in vitro* tissue models that accurately recapitulate the biology and physiology of organs in the human body for evaluation of nanomedicine to promote the well-being of human life. Through the merger of tissue engineering technologies with individual-specific human cells, human organ (tumor) models can be fabricated that better mimic human physiology or pathology. By further integrating proper blood surrogate and scaling laws, such 'human-on-a-chip' systems have potential to function as a robust platform that both applies to fundamental studies of biological agents and diseases, as well as to predicting the effects of nanomedicine in humans.

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