Microscale Technologies and Modular Approaches for Tissue Engineering: Moving toward the Fabrication of Complex Functional Structures

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ABSTRACT Micro- and nanoscale technologies have emerged as powerful tools in the fabrication of engineered tissues and organs. Here we focus on the application of these techniques to improve engineered tissue architecture and function using modular and directed self-assembly and highlight the emergence of this new class of materials for biomedical applications.

Advances in the field of tissue engineering and regenerative medicine, as indicated by the clinical approval of skin, cartilage, vascular grafts, and bladder, have shown that simple connective tissues can be produced *in vitro* and used to treat patients.[1−3] Most engineered tissues have been generated by seeding cells in porous scaffolds derived from natural and synthetic polymers. These scaffolds create a three-dimensional (3D) environment that promotes cellular attachment, migration, proliferation, and differentiation. Despite these advances, a number of technical challenges are currently preventing the development of more complex organs such as the liver, heart, and kidney.[4] These include the inability to reproduce the physical (substrate stiffness, architecture) and chemical (cytokines, growth factors, cell−cell, cell−matrix) interactions surrounding the cells *in vivo* and the lack of a suitable blood vessel supply to ensure cell function in thick tissues.

The structure and organization of the extracellular matrix (ECM) components and the interactions between the cellular and soluble factors found in tissue surroundings are known to play a significant role in the physiologic function of tissues and organs. Therefore, it is important that scaffolds recreate this microenvironment to engineer tissues with appropriate function. However, many current approaches aiming at tissue and organ regeneration are not designed for optimized performance at such length scales. The challenge is to develop technologies that will enable the engineering of scalable constructs reproducing the cellular microenvironment found *in vivo*. These approaches, which will be of use for generating large, functional, and vascularized 3D structures, should enable control of the arrangement of microscopic structures, which is essential to achieve the adequate level of functionality in engineered tissues. Current strategies are moving toward bioinspired methods to produce physiologically relevant tissues and organs. Major efforts are directed toward the generation of increasingly sophisticated materials that can mimic native tissues with respect to both architecture and functionality.[5]

> The challenge is to develop technologies that will enable the engineering of scalable constructs reproducing the cellular microenvironment found *in vivo*.

Microscale technologies are currently studied as potential tools for addressing this issue. The cell-seeded scaffold, which has led to significant advances over the past three decades, is currently shifting from empirical approaches to precisely engineered systems.[6] Techniques such as soft

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Published online May 31, 2011
10.1021/nn201826d

lithography, bioprinting, micromolding, and photolithography have emerged as powerful techniques to generate scaffolds for tissue engineering.[7−9] Application of micro- and nanotechnologies to the biomedical field has already led to numerous advances, notably in the pharmaceutical and biotechnology industries.[10,11] Recent breakthroughs have resulted in tissue engineering scaffolds that replicate cell-scale complexities into 3D structures.[6] These features can be obtained by using various techniques ranging from decellularized tissues to the combination of microfabrication technologies with modular assembly, which aim to reproduce the cell microenvironment with a high level of fidelity.

Modular Assembly for the Engineering of Complex Tissues and Biomimetic Structures. The fabrication of 3D tissues, such as the liver, heart, and kidney, remains a great challenge for tissue engineers since they all represent highly complex organs with specialized functions. Comprising multiple cell types, an extensive vasculature, and an intricate architecture, they combine the requirement for adequate structure, perfusion, and function in order to perform their duty.[12,13] Multiple developmental studies have shown that simple physical and chemical cues can give rise to complex outcomes, underscoring the fact that organ design does not necessarily imply complexity.[14] From the geometry of shell formation to the branching architecture and diffusion of molecules into vascular systems, a common observation is that only a handful of simple governing rules regulate the morphogenesis of complicated systems. The field of biomimetics, based on these principles, uses biological developments as a source of technological innovation and ideas.[14] Self-assembly processes in nature are triggered by simple guidelines, such as the attempt of a system to minimize its surface energy, which result in the aggregation of smaller particles. Therefore, the formation of 3D tissues through self-assembly of small subunits is a process that could be used to generate many tissue-like structures. For example, nephrons in the kidney, muscle fibers, liver lobules, and pancreatic islets all represent repeating units that are assembled into coherent 3D structures to enable a desired tissue function. To meet these specifications, bottom-up or modular assembly approaches have emerged as means to engineer controlled architectures precisely. These approaches use various physical forces to drive the aggregation of microscale objects to generate complex architectures from the directed assembly of tissue building blocks.[15−17]

> We have developed a bottom-up approach to direct the assembly of cell-laden microgels to form in 3D tissue constructs with tunable microarchitecture and complexity.

Whitesides and co-workers have pioneered the mesoscale assembly of millimeter-scale objects into precisely defined 2D and 3D structures using the minimization of interfacial free energy at the liquid−liquid interface.[18,19] Inspired by these findings, we have developed a bottom-up approach to direct the assembly of cell-laden microgels to form 3D tissue constructs with tunable microarchitecture and complexity.[16] These cell-containing microgels can be engineered to regulate the cellular environment in a specific and "intelligent" fashion.[20−23] By using microtechnologies, it is possible to create patterns of multiple cell types as well as gradients of chemicals and signaling molecules across the hydrogel materials, thus enabling regulation of cell behavior within the scaffolding material.[6,20,24] Moreover, a range of fabrication methods can also be used to control the shape of the resulting microgels (Figure 1A) and to generate microscale units in a high-throughput fashion.[25] Therefore, the directed assembly of cell-laden microscale hydrogels may be useful in generating bioengineered functional tissues with precisely engineered physical, chemical, and biological properties.

In previous studies, our group has shown that the assembly of microgel units can be driven by the tendency of multiphase liquid systems to minimize surface area and free energy.[16] This thermodynamically driven assembly technique relies on the hypothesis that the hydrophilic properties of microgels, combined with the hydrophobic properties of the medium, can be used as the driving force to generate 3D structures.[26] Mechanical stability of these assemblies can be controlled by a secondary cross-linking reaction using light exposure. This scalable technique can be used to generate biomimetic, 3D tissue constructs. To create more complex tissues and organs displaying physiologic morphology, modular approaches are moving toward the directed assembly of functional microunits (Figure 1B−D).[27] For example, lock-and-key-shaped microgels can assemble in a more predictable manner within a multiphase reactor system to generate 3D structures. It is envisioned that once the engineered building blocks are assembled in an ordered state, they will be remodeled by the cells, integrate with the host vasculature, and function as an organ substitute.[28]

The main limitation of self-assembly approaches that rely on liquid−air and hydrophilic−hydrophobic interactions is the restricted number of shapes that can be generated at the interfaces of the different phases. The packing process of microgels requires hierarchical and

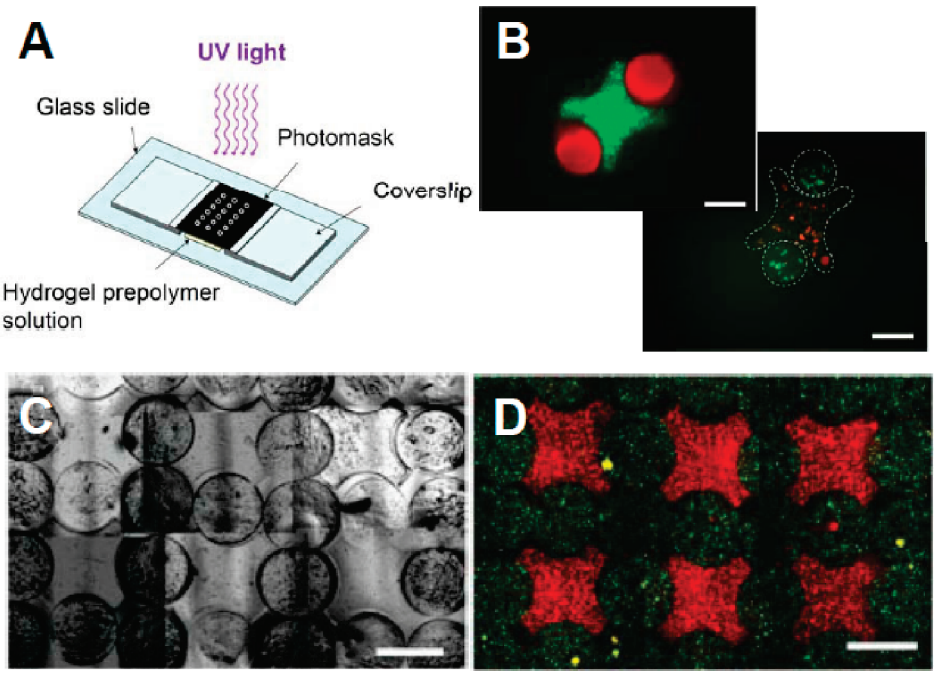

Figure 1. Directed assembly of microgels using a directed approach. (A) Schematic representation of a photolithographic approach. (B) Directed assembly of lock-and-key-shaped microgels stained with FITC-dextran and Nile red (top) or cell-laden microgels stained with Calcein AM and PKH26 (bottom). Scale bar: 200 μm. (C) Phase contrast and (D) fluorescence images of centimeter-scale engineered tissues obtained from the interface-directed assembly of cell-laden microgels. Scale bars: 1 mm. (A) Sequential assembly of cell-laden hydrogel constructs to engineer vascular-like microchannels. Reproduced with permission from ref 45. Copyright 2011 Wiley. (B) Directed assembly of cell-laden microgels for fabrication of 3D tissue constructs. Reproduced with permission from ref 16. Copyright 2008 National Academy of Sciences, USA. (C,D) Interface-directed self-assembly of cell-laden microgels. Reproduced with permission from ref 56. Copyright 2010 Wiley.

organizational driving forces that enable precise microgel placement and assembly, which are essential for recreating biomimetic tissue complexity. To address this issue, we have developed a technique whereby a solid surface acts as a template to direct the assembly process.[29] In this system, the solid surface of the template confines and restricts the microgels into a well-defined structure (Figure 2A,B). Due to the capillary forces of the prepolymer solution, microgels are able to pack densely around the surface of the template on which they are placed. The current challenge regarding this approach remains the ability to generate anisotropic 3D structures since the arrangement of units having distinct differences in their properties remains difficult. Since self-assembly processes are relying on physical and thermodynamic energy balances between states or phases, the optimal assembly of the microunits will depend on the properties of the material, as well as the nature of the driving forces used to trigger the aggregation of the building blocks. From a tissue engineering perspective, the assembly and packing of the microgels will need to be performed following stringent requirements. The control of chemical and physical interactions between the microgels will be essential for the development of desirable tissue function and stability of self-assembled hydrogel structures.[30] The development of modified interfaces using electrostatic charges or adhesion motifs could lead to more efficient bonding between the microgels, resulting in increased cohesion and stronger load-bearing capabilities. Consequently, the optimal physiologic performance of 3D engineered tissues will depend on the driving forces and the interfacial phenomena used to build these 3D structures because they will enable the fabrication of essential features such as the precise branching of perfusable vascular structure following microgel assembly.

Microengineering of 3D Branched Vasculature. A key limiting factor in the clinical translation of tissue engineering technologies is the inability to generate functional and thick tissues due to the absence of vascular structures in engineered tissues. Recent findings have demonstrated that endothelial cells involved in the angiogenic process not only form passive conduits to deliver nutrients and oxygen but also establish an instructive niche responsible for paracrine signaling stimulating organ regeneration, thus highlighting the importance of vascular structures in engineered tissues.[31] Previous strategies aiming at the engineering of vasculature have relied on the presence of endothelial cells, seeded or cocultured in the scaffold, to induce the release of growth factors and promote angiogenesis. This method was found to be adequate to form capillary-like structures that will ultimately connect with the host vasculature once the tissue is implanted *in vivo*.[32−34]

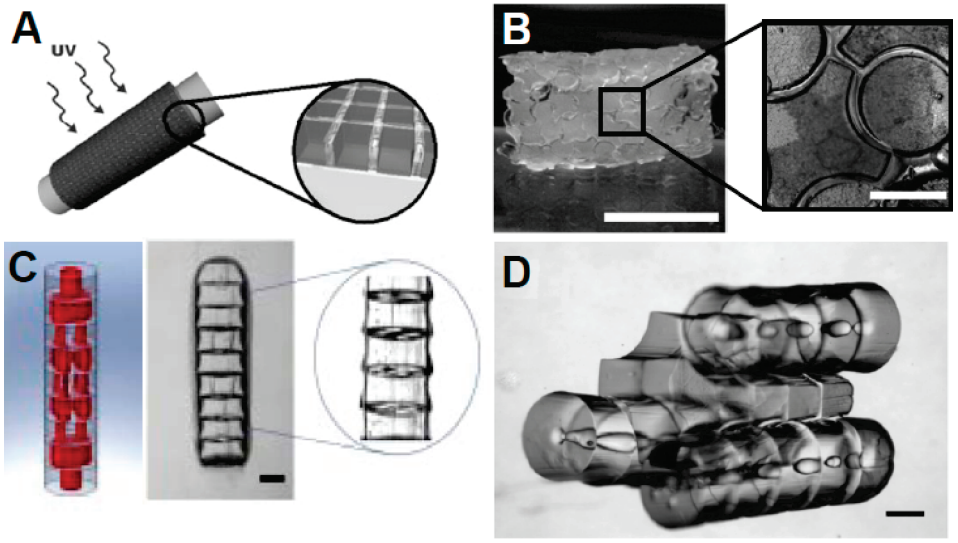

Figure 2. (A) Schematic diagram of the micromasonry assembly process. (B) Microgels are assembled on a template prior to a second cross-linking process. This resulted in a 3D structure composed of an assembly of microgels recapitulating the 3D structure of the template used for fabrication. Scale bar: 5 and 1 mm (magnification). (C) Design image of a microgel arrays assembled into tubular structures embedded with 3D branching lumens and actual phase image of the microgel assembly after secondary cross-linking. Scale bar: 500 μm. (D) Phase image of microgel assembly following a sequential and directed assembly process. Scale bar: 500 μm. (A,B) Micromasonry: construction of 3D structures by microscale self-assembly. Reproduced with permission from ref 29. Copyright 2010 Wiley. (C,D) Sequential assembly of cell-laden hydrogel constructs to engineer vascular-like microchannels. Reproduced with permission from ref 45. Copyright 2011 Wiley.

However, the amount of time required to generate proper vascularization and to achieve adequate transport of nutrients considerably reduces the efficiency of producing vascularized tissues and often leads to cell death and tissue necrosis.[4] Thus, this solution has not been able to generate organ-scale constructs *in vitro*.

> Microfabrication technologies—more specifically, microfluidic systems—have emerged as promising approaches to generate physiologically relevant vascular structures into tissue scaffolds.

Microfabrication technologies—more specifically, microfluidic systems—have emerged as promising approaches to generate physiologically relevant vascular structures into tissue scaffolds.[35] These approaches mostly rely on engineered channel networks fabricated in biodegradable polymers.[36−40] However, most of the vascularized systems are built using top-down approaches and are generally found in planar or stacked 2D structures.[41] Although previous work has shown that microscale cell-laden channels can be engineered *in vitro*, it is particularly difficult to branch multidimensional channels consecutively in 3D.[42] Techniques such as direct ink writing and omnidirectional printing have recently been developed to create 3D vascular structures.[43,44] Despite enormous potential, these approaches will require further improvement to enable the control of the tissue structures surrounding the vascular channels. However, modular assembly techniques can be rationally engineered using cell-laden microgels produced by photolithography. Photolithography and self-assembling systems represent novel approaches to building biomimetic vascular-like structures for tissue engineering and *in vitro* models. Our group has developed a simple approach to direct the assembly of cell-laden microengineered hydrogels embedded with vascular-like microchannels having circular lumens.[45] The sequential assembly of hydrophilic hydrogels, performed in a biphasic reactor, resulted in a 3D structure with multilevel interconnected branching vasculature (Figure 2C). In addition to the directed assembly of the microgels, smooth muscle cells and endothelial cells were encapsulated in the 3D construct and remained viable for an extended period of time.[45] Compared to previous work, this sequential assembly technique of vascularized units is a step forward in our ability to control the relative spatial arrangement of the building blocks and the architecture of the 3D assembly.[16,17] In a continuation of this work, the long-term perfusion of these capillary networks will be investigated. The engineering of organs, which requires biological complexity including endothelial cells to improve vascular activity as well as other specialized cell types required for tissue function and

integrity, will benefit from the sequential assembly process enabling the fabrication of 3D constructs containing multiple cell types with defined architectures and functions. Given these results, it appears that modular tissue engineering may be useful in controlling the microenvironment of large and vascularized 3D structures, more specifically for building scaffolds requiring cell-scale precision (Figure 2D).

Microscale Bioassays and Validation Tools for Engineered Tissue Functionality. It has been shown that individual cell-containing microgels can be fabricated and hierarchically assembled into 3D structures, leading to organized and branched architectures,[16,45] However, the characterization of the physiologic functionality of these cell-laden microgels and the macroscale structure resulting from their assembly still needs to be clearly demonstrated. To generate functional tissues, individual units will have to display appropriate properties prior to their incorporation into the 3D structure. The assembly may also have to demonstrate adequate physiologic functionality, as well as perfusion capabilities and structural strength. The development of new classes of biosensors that will assess the functionality of both the microgels and their assembly will be of tremendous importance in enabling this technology. Microscale technologies have been used to develop numerous tools to investigate cell−cell and cell−microenvironment interactions *in vitro*.[6] It has also been shown that microfabricated systems can be used as sensors in microdevices.[46] For example, microelectromechanical systems (MEMS) platforms have been incorporated into a variety of biosensors and analytical tools due to their miniature size and ultrahigh sensitivity.[47−50] So far, most of these devices have been designed for *in vitro*, lab-on-a-chip use. *In vivo* considerations such as biomechanics, distribution and removal of soluble factors, and toxin level detection have been the focus of only a few studies using microfabricated implants.[46] Engineered tissues incorporating biologically relevant and implantable microdevices that could monitor and validate tissue function would greatly benefit from the ability to detect important physiological parameters found *in vivo*. This perspective could also considerably improve the design of engineered tissues through a feedback loop of implementation provided by the readings recorded by these biosensors.

Microengineering the Stem Cell Niche. The engineering of the cell microenvironment has been shown to have a strong influence on the regulation of stem cell fate.[51] The combination of microfabrication and stem cell technologies could be used to dictate cell and tissue behavior during the fabrication process *in vitro* and to trigger or to activate full functionality following implantation *in vivo*.[20] Stem cells represent a potentially unlimited source of cells for tissue engineering and regenerative medicine and can be used to produce multiple engineered tissues using a single cell type.[52] Nonetheless, there are significant issues in the control, efficiency, and reproducibility of the differentiation process that need to be understood to fully realize the potential of this technology. Most recent approaches to direct stem cell fate are based on mimicking *in vivo* developmental processes by using spatial and temporal cues as well as various extrinsic cues such as soluble factors and basement membrane constituents.[53] Therefore, microscale approaches could be used to microengineer artificial stem cell niches, to study cell−environment interactions *in vitro* and to dictate cell fate upon implantation *in vivo*. This could be especially important for organ engineering, where the tissue may not only perform a load bearing or barrier function but may also perform an essential physiologic duty. A number of researchers have shown that microtechnologies can be used to control the differentiation of stem cells by mimicking the anisotropy of the stem cell niche.[54,55] The combination of stem cell technology with modular approaches could help to control the restoration of tissue morphology and function since microtechnologies can be used to engineer the bioactivity, shape, and localization of the substrate on which cells attach. Building scaffolds and devices mimicking the stem cell niche and controlling the structural anisotropy and biological variations at the microscale level could optimize the cell−material interactions and therefore increase the success rate of their utilization for tissue regeneration and integration.

CONCLUSIONS AND PROSPECTS

Although tissue engineering has been described as the next generation of available treatment to replace and to regenerate organs, this technology has not yet fully realized its potential. This can be explained by the fact that engineered tissues previously developed were simple and lacked the complexity associated with many native tissues. Organ function and regeneration is highly dependent on proper spatial placement and arrangement of multiple single units, as well as on inductive and adequate signaling throughout the structure. Recent advances in microtechnologies have increased our capability to engineer functional tissues for therapeutic applications. The design of new methods that enable the directed self-assembly of microgels into 3D configurations composed of microfluidic branched structures has shown significant potential for tissue engineering applications. Moreover, the emergence of modular assembly is currently enabling the development of a new class of functional and instructive engineered tissues. The success of these novel techniques promises to address current challenges, such as nutrient and oxygen

transport and vascularization, and will ultimately translate into functional and readily available organs for transplantation. In addition, microtechnologies may also lead to the development of new biosensors and biomimetic microdevices. This convergence of multiple research fields, ranging from biomaterials to microfabrication and stem cell biology, is highly promising in leading to the generation of engineered biological systems for clinical applications.

Acknowledgment. This work was supported by the National Institutes of Health (EB008392; HL092836; HL099073; EB009196; DE019024), National Science Foundation (DMR0847287), the Institute for Soldier Nanotechnology, the Office of Naval Research, and the U.S. Army Corps of Engineers. R.G. holds a postdoctoral fellowship from FQRNT.

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