

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

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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–ECM) 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

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.

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 approaches 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 approach, 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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135 lithography, bioprinting, micro-
 136 molding, and photolithography
 137 have emerged as powerful ap-
 138 proaches to generate scaffolds for
 139 tissue engineering.^{7–9} Application
 140 of micro- and nanotechnologies to
 141 the biomedical field has already led
 142 to numerous advances, notably in
 143 the pharmaceutical and biotechnol-
 144 ogy industries.^{10,11} Recent break-
 145 throughs have resulted in tissue
 146 engineering scaffolds that replicate
 147 cell-scale complexities into 3D
 148 structures.⁶ These features can be
 149 obtained by using various techni-
 150 ques ranging from decellularized
 151 tissues to the combination of micro-
 152 fabrication technologies with modu-
 153 lar assembly, which aim to reproduce
 154 the cell microenvironment with a
 98 high level of fidelity.

99 **Modular Assembly for the Engineering**
 100 **of Complex Tissues and Biomimetic Struc-**
 101 **tures.** The fabrication of 3D tissues,
 102 such as the liver, heart, and kidney,
 103 remains a great challenge for tissue
 104 engineers since they all represent
 105 highly complex organs with specia-
 106 lized functions. Comprising multiple
 107 cell types, an extensive vasculature,
 108 and an intricate architecture, they
 109 combine the requirement for ade-
 110 quate structure, perfusion, and
 111 function in order to perform their
 112 duty.^{12,13} Multiple developmental
 113 studies have shown that simple
 171 physical and chemical cues can give
 172 rise to complex outcomes, under-
 173 scoring the fact that organ design
 174 does not necessarily imply
 175 complexity.¹⁴ From the geometry
 176 of shell formation to the branching
 177 architecture and diffusion of mol-
 178 ecules into vascular systems, a com-
 179 mon observation is that only a
 180 handful of simple governing rules
 181 regulate the morphogenesis of
 182 complicated systems. The field of
 183 biomimetics, based on these princi-
 184 ples, uses biological developments
 185 as a source of technological innova-
 186 tion and ideas.¹⁴ Self-assembly pro-
 187 cesses in nature are triggered by
 188 simple guidelines, such as the at-
 189 tempt of a system to minimize its
 190 surface energy, which result in the
 191 aggregation of smaller particles.

Therefore, the formation of 3D tis-
 sues 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 re-
 present repeating units that are as-
 sembled into coherent 3D structures
 to enable a desired tissue function.
 To meet these specifications, bot-
 tom-up or modular assembly ap-
 proaches have emerged as means
 to engineer controlled architectures
 precisely. These approaches use var-
 ious physical forces to drive the
 assembly of microscale objects to
 generate complex architectures from
 the directed assembly of tissue build-
 ing 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 pre-
 cisely 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 bot-
 tom-up approach to direct the as-
 sembly of cell-laden microgels to
 form in 3D tissue constructs with
 tunable microarchitecture and
 complexity.¹⁶ These cell-containing
 microgels can be engineered to reg-
 ulate the cellular environment in a
 specific and “intelligent” fashion.^{20–23}
 By using microtechnologies, it is
 possible to create patterns of multi-
 ple 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
 approaches can also be used to
 control the shape of the resulting
 microgels (Figure 1A) and to gener-
 ate microscale units in a high-
 throughput fashion.²⁵ Therefore,
 the directed assembly of cell-laden
 microscale hydrogels may be useful
 in generating bioengineered func-
 tional tissues with precisely engi-
 neered 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 thermody-
 namically driven assembly techni-
 que relies on the hypothesis that
 the hydrophilic properties of micro-
 gels, combined with the hydropho-
 bic properties of the medium, can
 be used as the driving force to gen-
 erate 3D structures.²⁶ Mechanical
 stability of these assemblies can be
 controlled by a secondary cross-
 linking reaction using light expo-
 sure. This scalable approach can be
 used to generate biomimetic, 3D
 tissue constructs. To create more
 complex tissues and organs display-
 ing physiologic morphology, modu-
 lar approaches are moving toward
 other approaches to direct the as-
 sembly of these functional microu-
 nits (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 engi-
 neered building blocks are as-
 sembled in an ordered state, they
 will be remodeled by the cells, in-
 tegrate with the host vascula-
 ture, and function as an organ
 substitute.²⁸

The main limitation of self-assem-
 bly approaches that rely on liquid–
 air and hydrophilic–hydrophobic
 interactions is the restricted num-
 ber of shapes that can be generated
 at the interfaces of the different
 phases. The packing process of

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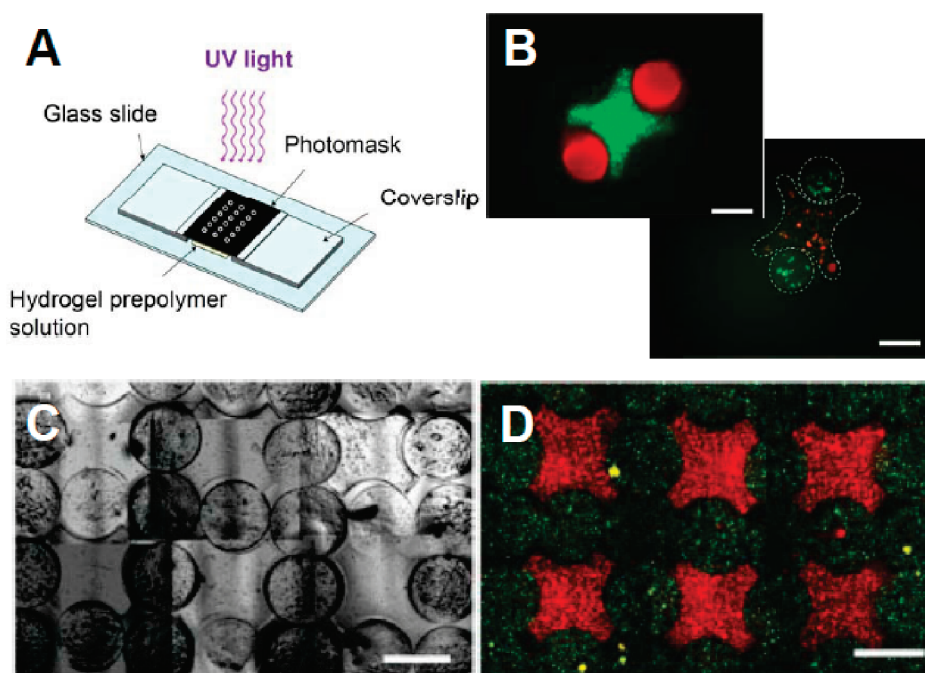


Figure 1. Directed assembly of microgels using a directed approach. (A) Schematic representation of a photolithographic approach. (B) Directed assembly of lock-and-key-shaped microgels stained with FITC-dextran and Nile red (top) or cell-laden microgels stained with Calcein AM and PKH26 (bottom). Scale 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.

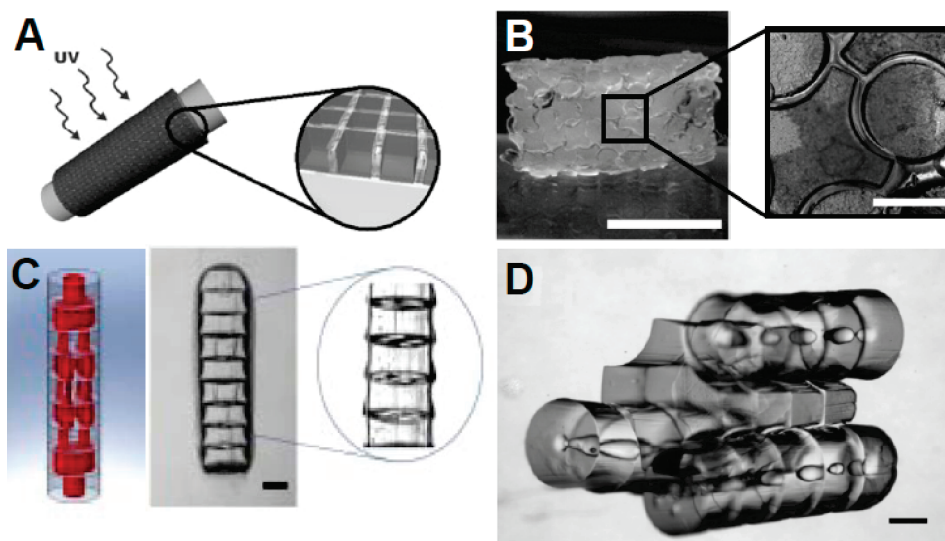


Figure 2. (A) Schematic diagram of the micromasonry assembly process. (B) Microgels are assembled on a template prior to a second cross-linking process. This resulted in a 3D structure composed of an assembly of microgels recapitulating the 3D structure of the template used for fabrication. Scale bar: 5 and 1 mm (magnification). (C) Design image of a microgel array 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.

252 microgels requires hierarchical and
253 organizational driving forces that
254 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

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315 process.²⁹ In this system, the solid
 316 surface of the template confines
 317 and restricts the microgels into a
 F2 318 well-defined structure (Figure 2A,B).
 319 Due to the capillary forces of the pre-
 320 polymer solution, microgels are able
 321 to pack densely around the surface
 322 of the template on which they are
 323 placed. The current challenge re-
 324 garding this approach remains the
 325 ability to generate anisotropic 3D
 326 structures since the arrangement of
 327 units having distinct differences in
 328 their properties remains difficult. Since
 329 self-assembly processes are relying on
 330 physical and thermodynamic energy
 331 balances between states or phases,
 332 the optimal assembly of the micro-
 333 units will depend on the properties
 334 of the material, as well as the nature
 335 of the driving forces used to trigger
 336 the aggregation of the building
 337 blocks. From a tissue engineering
 338 perspective, the assembly and
 339 packing of the microgels will need
 340 to be performed following stringent
 341 requirements. The control of chemical
 342 and physical interactions between
 343 the microgels will be essential for
 287 the development of desirable tissue
 288 function and stability of self-as-
 289 sembled hydrogel structures.³⁰ The
 290 development of modified interfaces
 291 using electrostatic charges or adhe-
 292 sion motifs could lead to more effi-
 293 cient bonding between the microgels,
 294 resulting in increased cohesion and
 295 stronger load-bearing capabilities.
 296 Consequently, the optimal physio-
 297 logic performance of 3D engi-
 298 neered tissues will depend on the
 299 driving forces and the interfacial
 300 phenomena used to build these
 301 3D structures because they will en-
 302 able the fabrication of essential fea-
 303 tures such as the precise branching
 304 of perfusable vascular structure fol-
 305 lowing microgel assembly.

362 **Microengineering of 3D Branched Vas-**
 363 **culature.** A key limiting factor in the
 364 clinical translation of tissue engi-
 365 neering technologies is the inability
 366 to generate functional and thick
 367 tissues due to the absence of vascular
 368 structures in engineered tissues.
 369 Recent findings have demonstrated
 370 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 or-
 gan regeneration, thus highlighting
 the importance of vascular struc-
 tures in engineered tissues.³¹ Pre-
 vious strategies aiming at the
 engineering of vasculature have re-
 lied on the presence of endothelial
 cells, seeded or cocultured in the
 scaffold, to induce the release of
 growth factors and promote angio-
 genesis. This method was found to
 be adequate to form capillary-like
 structures that will ultimately con-
 nect with the host vasculature once
 the tissue is implanted *in vivo*.^{32–34}
 However, the amount of time re-
 quired to generate proper vascular-
 ization and to achieve efficient
 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 sys-
 tems—have emerged as promising
 approaches to generate physiologi-
 cally relevant vascular structures
 into tissue scaffolds.³⁵ These ap-
 proaches mostly rely on engineered
 channel networks fabricated in bio-
 degradable polymers.^{36–40} However,

371 most of the vascularized systems
 372 are built using top-down appro-
 373 aches and are generally found in
 374 planar or stacked 2D structures.⁴¹
 375 Although previous work has shown
 376 that microscale cell-laden channels
 377 can be engineered *in vitro*, it is
 378 particularly difficult to branch multi-
 379 dimensional channels consecutively
 380 in 3D.⁴² Techniques such as
 381 direct ink writing and omnidirec-
 382 tional printing have recently been
 383 developed to create 3D vascular
 384 structures.^{43,44} Despite enormous
 385 potential, these approaches will
 386 require further improvement to
 387 enable the control of the tissue
 388 structures surrounding the vascular
 389 channels. However, modular assem-
 390 bly techniques can be rationally en-
 391 gineered using cell-laden microgels
 392 produced by photolithography.
 393 Photolithography and self-assem-
 394 bling systems represent novel ap-
 395 proaches to building biomimetic
 396 vascular-like structures for tissue
 397 engineering and *in vitro* models.
 398 Our group has developed a simple
 399 approach to direct the assembly of
 400 cell-laden microengineered hydro-
 401 gels embedded with vascular-
 402 like microchannels having circular
 403 lumens.⁴⁵ The sequential assembly
 404 of hydrophilic hydrogels, performed
 405 in a biphasic reactor, resulted in a
 406 3D structure with multilevel inter-
 407 connected branching vasculature
 408 (Figure 2C). In addition to the direc-
 409 ted assembly of the microgels,
 410 smooth muscle cells and endothe-
 411 lial cells were encapsulated in the
 412 3D construct and remained viable
 413 for an extended period of time.⁴⁵
 414 Compared to previous work, this
 415 sequential assembly technique of
 416 vascularized units is a step forward
 417 in our ability to control the relative
 418 spatial arrangement of the building
 419 blocks and the architecture of the
 420 3D assembly.^{16,17} In a continuation
 421 of this work, the long-term perfu-
 422 sion of these capillary networks will
 423 be investigated. The engineering of
 424 organs, which requires biological
 425 complexity including endothelial
 426 cells to improve vascular activity as
 427 well as other specialized cell types

485	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.	542
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491	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).	548
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498	Microscale Bioassays and Validation Tools for Engineered Tissue Functionality.	555
499	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 <i>in vitro</i> . 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 <i>in vitro</i> , lab-on-a-chip use. <i>In vivo</i> considerations such as biomechanics, distribution and removal of	556
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	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 <i>in vivo</i> . 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 <i>in vitro</i> and to trigger or to activate full functionality following implantation <i>in vivo</i> . 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 <i>in vivo</i> developmental processes by using spatial and temporal cues as well as various extrinsic cues such as soluble factors and extracellular matrix and basement membrane constituents. ⁵³ Therefore, microscale approaches could be used to microengineer artificial stem-cell niches, to study cell–environment interactions <i>in vitro</i> , and to dictate cell fate upon implantation <i>in vivo</i> . 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.

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