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Advanced micro- and nanofabrication technologies for tissue engineering

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The ability of the living body to heal and regenerate itself following trauma is astonishing. Numerous events of repair and regeneration occur during our lifetime, most of which we are never aware of. Unfortunately, in some cases, the injury or defect cannot be adequately repaired solely by nature and medical intervention is required.

Tissue engineering is a multidisciplinary field of science that integrates knowledge from engineering, biology, chemistry and medicine [1]. It focuses on the development of functional tissues or organs that can be used to repair or substitute their defected, injured or diseased natural counterparts. As opposed to superficial growth of cells in a culture dish, cells that populate a functional tissue are usually integrated in a complex, three-dimensional (3D) architecture of extracellular matrix (ECM) and vasculature. With the recent advances in cellular biology research, it becomes increasingly clear that the micro- and nanoscale cues in the surrounding microenvironment of the cells have a profound effect on their growth, differentiation, morphology and metabolic state [2, 3]. At a higher hierarchical level, the cues provided by the ECM can direct the morphogenesis of the whole tissue. When they recognized the importance of chemical and biomechanical signals in tissue organization, scientists began to develop materials and methods for mimicking the natural cellular microenvironment.

In this special section of *Biofabrication*, we bring together original research articles from top scientists in the field of biomaterials and regenerative medicine. Each presents state-of-the-art micro- and nanotechnologies and developments in biofabrication for tissue engineering.

Great emphasis is currently given to the biofabrication of biomimetic 3D scaffolds that replicate the architecture and mechanical properties of the natural ECM. Fabrication

methods such as self-assembly, particulate-leaching, freeze-drying, phase-separation and electrospinning were developed to produce biodegradable, porous polymeric scaffolds from natural or synthetic materials. The fabricated scaffolds can also be further modified to improve cell adherence or present and deliver chemical cues that can 'guide' the cells toward their desired fate (e.g. proliferation, differentiation, migration, regulation of a specific cellular function, etc). The porous scaffold is then populated with cells that secrete a structural protein network and thus create their own ECM. This traditional approach, which is called 'top-down' or 'solid-scaffold-based tissue engineering', is demonstrated in this special issue by Szymanski and Feinberg [4]. In their paper, a method for the fabrication of an alginate and alginate-fibrin-based microfibrous structure with tunable geometries is described. The authors used photolithography in order to fabricate an elastic stamp for imprinting the biomaterial onto a thermally sensitive sacrificial layer. Subsequently, the material was cross-linked and the microstructures were released from the support. The resulting fine fabric supported the polarization of myoblasts and the formation of interconnected cell strands. Another example of using a lithographic method for fabrication of biodegradable scaffold is reported by Kim *et al* [5]. The group used UV-assisted capillary force lithography to fabricate nanopatterned cell culture substrates for vascular tissue engineering. By varying the amount and ratio of the constituents of poly(ethylene glycol)-gelatin methacrylate hydrogel, the elasticity and degradation rate of the composite structure could be tuned. Importantly, in comparison to an unpatterned substrate, endothelial cells showed better attachment and native vascular cell-like morphology when seeded on the patterned scaffold. The pattern also promoted rapid migration and anisotropic organization of the cells. Together, these two lithography-assisted techniques could be applied in a wide range of tissue engineering applications when a controllable and precise fabrication of fine geometries is required.

In order to closely mimic the natural 3D extracellular environment and attain improved substrate-specific cell adhesion and spreading, a unique composite scaffold made of biofunctionalized, electrospun fibers and a hydrogel was fabricated by Schulte et *al* [6]. Following a short period of pre-culturing on the functionalized fibers, the cell-seeded matrix was covered with a protective semi-synthetic hyaluronic acid-based hydrogel that further supported cell proliferation and spreading along the embedded fibers. While embedding an originally superficial cell-seeded fibrous matrix in a hydrogel represents an intriguing strategy for mimicking the natural 3D environment of the cells, the native architecture of the ECM may also be mimicked by spatial fabrication of the fibers themselves. For instance, Park et al [7] developed a method to fabricate fibrous, 3D cell-laden alginate scaffolds using a single microfluidic platform. The authors showed the capability of their set-up to control the shape, volume and porosity of the scaffolds while preserving their delicate structure. Moreover, the porous nature of the scaffolds, which permits a high diffusion rate, was found to be superior in maintaining cell viability when compared to a 3D alginate non-fibrous block.

Another creative strategy for the fabrication of 3D fibrous scaffolds was developed by Park et al [8]. The authors describe a hybrid process for thermal fusion of uniaxially oriented electrospun nanofibers by algorithm-directed molten polymer deposition. The aligned fibers were bound together by thermally extruded microstructures to create 2D mats that can be

used as one-layer fibrous substrates or can be stacked to produce 3D multilayer constructs. The feasibility of these composite scaffolds for application in muscle tissue engineering was demonstrated by obtaining viable and highly aligned cultured myoblasts.

Overall, the aforementioned studies describe three elegant strategies for fabricating fibrous 3D scaffolds. The morphological and biochemical resemblance of these scaffolds to the native ECM, together with their biocompatibility and biodegradability, underline their potential to serve as biomimetic substrates for tissue engineering applications.

The solid-scaffold-based approach of populating a pre-fabricated scaffold with cells was also utilized by Zieber et al [9]. Recognizing the importance of pre-vascularization of engineered tissues for maintenance of cell viability and for effective integration with the host, the researchers have used a CO2 laser engraving system to create an array of microscale channels within alginate macroporous scaffolds. The channels were then decorated with adhesion peptides and angiogenic factors to create a blood-vessel-supporting microenvironment. Upon sequential seeding of endothelial cells, cardiomyocytes and fibroblasts, the unique architecture and the biochemical cues promoted the generation of vessel-like networks within an engineered cardiac patch.

Another important aspect for engineering functional cardiac tissues is the structural and functional anisotropy of the tissue. In order to control these parameters, Bian et al [10] have applied a high aspect ratio soft lithography technique to generate network-like tissue patches composed of cardiomyocytes. The authors have shown that the alignment of the cells and secreted ECM proteins can be enhanced by extending the transverse diameter of the elliptical pores that crossed the patch networks. The improved alignment resulted in increased anisotropy of the action potential propagation and augmented contractile forces.

While synthetic scaffolds made of biocompatible materials can be fabricated to closely mimic the ECM structure, they still lack much of the fine, complex architecture and biochemical cues that can be found in the native ECM. In a study by Shevach et *al* [11], the omentum, a double sheet of peritoneum, was manipulated to serve as a natural, autologous scaffold for engineering functional cardiac tissue. It was found that the biochemical and mechanical properties of the processed omental tissue could support the survival and assembly of cardiac cells into a contractile patch. The patch could then be populated with endothelial cells to generate a vascularized tissue for better integration with the host. Since the omentum can easily and safely be extracted from patients, this approach might be utilized to engineer autologous patches. To prove this concept, the authors also showed that the omentum-based matrices can support the growth of induced pluripotent and mesenchymal stem cells, which in theory can also be isolated from the same patient.

Whereas fabrication of ECM-like scaffolds is a key process in tissue engineering for regenerative medicine, it can also be utilized for construction of 3D tissue models for more basic research. Ock and Li [12] have used a laser foaming technique to fabricate an array of micro-scale porous polylactic acid scaffolds for high throughput, tissue-based biomedical assays. The authors have studied the effects of the process parameters on the resulting internal architecture of the scaffolds and demonstrated enhanced cell viability within the

scaffolds. The fabricated microarray represents a more biologically relevant model for drug screening than the common 2D cultures.

While the ‘top-down’ approach is mostly suitable for engineering thin, non-vascularized artificial tissues, the construction of thicker, complex tissues with micro- and nanoscale architectural characteristics is still a challenge. The emerging ‘bottom-up’ or ‘modular’ approach aims to overcome this issue. This concept is based on the generation of microscale tissue building blocks that incorporate complex artificial micro- and nano-architectures resembling those of a native tissue. The tissue components can be fabricated by using various methods such as self-assembled cell aggregates, generation of cell sheets and fabrication of cell-laden hydrogels. These building blocks are then assembled to form a large tissue construct using methods such as random packing, stacking of layers and 3D bioprinting. For example, in their article Lee *et al* [13] used a 3D printing method for reconstruction of the external ear. The authors encapsulated chondrocytes and adipocytes in an alginate hydrogel and dispensed them into ear-shaped structures made of sacrificial-layer-supported polycaprolactone. Using this strategy, both the shape and the composition of the ear were faithfully mimicked. Importantly, the adipocytes and chondrocytes survived the printing process, proliferated and showed both adipogenesis and chondrogenesis, respectively. Without doubt, this study has the potential to broaden the current arsenal of practical methods used in reconstructive medicine, especially in cases when the target tissue or organ has a complex structure.

Another example of harnessing the power of 3D printing for tissue fabrication was reported by Bertassoni et al [14]. The authors demonstrate a strategy for 3D bioprinting using a modified set-up that uses a photolabile cell-laden methacrylated gelatin as ‘bioink’ for fabrication of pre-polymerized hydrogel fibers. After assessing the effect of the biopolymer concentration and cell density on the fabrication process, the authors proved the ability of their method to fabricate and control macroscale architectures and to support the viability of the printed cells.

A different strategy of 3D bioprinting involves the fabrication of ‘scaffold-free’ constructs using tissue spheroids as bioink. In this process, the basic building blocks of the tissue are spherical bodies composed of spontaneous self-assembled cells. Upon deposition in a pre-defined architecture, the spheroids fuse into a 3D tissue structure. In their paper, Tan *et al* [15] describe a technique for direct 3D mold printing for fabrication of scaffold-free tissue engineering constructs. Using algorithm-directed deposition of alginate micro-droplets on a calcium-containing substrate, the group managed to construct hydrogels with defined 3D architecture. The resulting structure then served as a mold into which tissue spheroids were deposited and eventually fused together to form an artificial, tissue-like structure. The promising results from these three studies demonstrate the emerging role of 3D printing technology as an effective means of implementing the modular approach. By performing a precise, algorithm-guided deposition of cells and biomaterials, ‘bottom-up’ fabrication of composite constructs has become feasible.

Whether the fabrication method of choice is based on the ‘top-down’ or ‘bottom-up’ approach, the subsequent step is usually an incubation period in a bioreactor to support the

organization and maturation of the construct into a functioning tissue. In this issue Miklas et al [16] report on the development of a bioreactor that offers both mechanical and electrical stimuli to engineered cardiac tissues and allows the researcher to study the physiology of the patch by providing on-line measurements of contraction force.

In summary, the articles in this special section demonstrate selected current advances in the fabrication of biomaterials in the context of tissue engineering. The recent achievements in this evolving, multidisciplinary field bring the scientific community another step toward the goal of faithfully imitating the fine and complex architecture of a functional human tissue. Finding the most appropriate cell source and engineering a thick, vascularized tissue are still a challenge. Nevertheless, the intense work and vast efforts that are being invested in research and development of artificial tissues for treatment of damaged or malfunctioning organs give hope to many patients that currently relay on organ transplantation from scarce donors.

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