Hindawi Publishing Corporation
Journal of Nanomaterials
Volume 2015, Article ID 213521, 14 pages
http://dx.doi.org/10.1155/2015/213521

Review Article

3D Nanoprinting Technologies for Tissue Engineering Applications

Jin Woo Lee

Department of Molecular Medicine, School of Medicine, Gachon University, Songdo-Dong, Yeonsu-gu, Incheon 406-840, Republic of Korea

Correspondence should be addressed to Jin Woo Lee; jwlee@gachon.ac.kr

Received 5 August 2015; Revised 21 October 2015; Accepted 22 October 2015

Academic Editor: Ramaswamy Narayanan

Tissue engineering recovers an original function of tissue by replacing the damaged part with a new tissue or organ regenerated using various engineering technologies. This technology uses a scaffold to support three-dimensional (3D) tissue formation. Conventional scaffold fabrication methods do not control the architecture, pore shape, porosity, or interconnectivity of the scaffold, so it has limited ability to stimulate cell growth and to generate new tissue. 3D printing technologies may overcome these disadvantages of traditional fabrication methods. These technologies use computers to assist in design and fabrication, so the 3D scaffolds can be fabricated as designed and standardized. Particularly, because nanofabrication technology based on two-photon absorption (2PA) and on controlled electrospinning can generate structures with submicron resolution, these methods have been evaluated in various areas of tissue engineering. Recent combinations of 3D nanoprinting technologies with methods from molecular biology and cell dynamics have suggested new possibilities for improved tissue regeneration. If the interaction between cells and scaffold system with biomolecules can be understood and controlled and if an optimal 3D environment for tissue regeneration can be realized, 3D nanoprinting will become an important tool in tissue engineering.

1. Introduction

Tissue engineering recovers an original function of tissue by replacing the damaged part with a new tissue or organ that has been regenerated using various engineering technologies. Tissue engineering is composed of three essential components: cell, biomolecules, and scaffold. Scaffolds supply an artificial structure that can support three-dimensional (3D) tissue formation. Cells seeded into the scaffold proliferate and differentiate, and biomolecules in the culture medium accelerate these processes. When the three components are suitably combined, a tissue is regenerated successfully. To achieve the tissue regeneration, scaffolds should be biocompatible and must have adequate pore size, high interconnectivity, and appropriate mechanical properties. Biodegradability should also be considered, because scaffolds should preferably be absorbed by the body, thereby eliminating the need for surgical removal.

Initially, most scaffolds were fabricated by traditional methods [1–13] such as gas foaming, freeze-drying, particulate leaching, phase separation/inversion, and fiber bonding. However, because those methods do not control the architecture, pore shape, porosity, or interconnectivity of the scaffold, they did not adequately stimulate cell growth and tissue generation.

3D printing technology using computer-aided design (CAD) and computer-aided manufacturing (CAM) may overcome these disadvantages. Because these methods use computer software to design and fabricate the scaffolds, their internal architectures such as the pore size, pore shape, porosity, and the interconnectivity of the scaffolds can be freely controlled. In addition, computer-aided 3D printing can produce reproducible constructs, so it enables standardization of scaffolds. This standardization eliminates variability of the inner architecture among scaffolds, so it improves the repeatability and reliability of experiments. These technologies can also fabricate customized scaffolds for patients.

Various 3D printing technologies [14–28] including stereolithography, deposition modeling, inkjet printing, selective laser sintering, and electrospinning technology have been

developed. Those technologies have been widely used in studies of regeneration of tissues such as bone, cartilage, ligament, muscle, skin, and neurons and of organs such as trachea, liver, kidney, and heart. 2PP and electrospinning can fabricate constructs to submicron precision. Therefore, this review will provide the present condition of scaffold fabrication using these 3D printing technologies.

2. 3D Nanoprinting for Tissue Engineering

2.1. Two-Photon Absorption (2PA) Based 3D Printing. Stereolithography (SL) was developed independently by Kodama [35] and Nakai and Marutani [36] in the 1980s. 3D Systems Inc. sold a commercialized SL system for the first time. SL uses an ultraviolet (UV) laser beam to irradiate the surface of a liquid photopolymer, causing it to solidify. Many scanned UV laser lines are overlapped on the surface to solidify a specified cross-sectional area; many such cross-sectional areas are accumulated step by step to form the desired 3D shape. Microstereolithography (MSTL) uses the same fabrication mechanism as SL but uses optical components to reduce the diameter of the laser beam to a few micrometers [37]. The laser beam is passed through a beam expander and focusing lens (Figure 1(a)) and then solidifies a very small area of the liquid photopolymer surface. MSTL enables fabrication of 3D freeform structures at micrometer scales.

Two-photon polymerization (2PP) is a laser-based 3D printing technique that uses two-photon absorption (2PA) [38, 39]. 2PA can be used to induce laser-based erosion by photoreaction of an irradiated material and ablation by an intense laser. In 2PP, a laser is used to trigger a chemical reaction that causes polymerization of a photosensitive material, as in SL and MSTL. However, unlike the single-photon polymerization process of SL and MSTL, 2PP allowed electron transitions over excited energy levels for the polymerization process, when an atom absorbs two photons simultaneously (~femtosecond level) (Figure 1(b)). For instance, when a specific photoinitiator that reacts at wavelength $\lambda = 400\,\text{nm}$ simultaneously absorbs two photons with $\lambda = 800\,\text{nm}$, their energies add up to equal the energy of one photon with $\lambda = 400\,\text{nm}$ and thus initiate the polymerization process. Photopolymerization that is triggered by nonlinear excitation happens at the focal point, but other regions are not affected by the laser energy. This phenomenon has the potential to reduce solidification resolution to below the diffraction limit of the applied light. In addition, the movement of the laser focal point and solidification inside the liquid photopolymer guarantee the fabrication of a 3D product. Therefore, 2PP currently has the highest resolution of all 3D printing techniques.

By combining CAD and CAM, the inner architecture of the structure can be precisely controlled. As a result of these features, 2PP offers great potential for the fabrication of appropriate scaffolds for tissue engineering. In addition, development of photodegradable polymer has enabled a two-photon erosion process, and modulation of a two-photon pulse laser has produced an ablation technique with submicron resolution.

2.1.1. Two-Photon Polymerization Technology for Tissue Engineering. By exploiting the high resolution of 2PP, many researchers have focused on the realization of 3D environments for cell adhesion and proliferation. Mostly, this research concentrated on methods to fabricate the 3D scaffold, which is an essential environment to regenerate damaged tissue.

Koroleva et al. [40] used a combination of 2PP and micromolding to fabricate 3D fibrin scaffolds with tightly controllable pore sizes and interconnections. The authors used 2PP to fabricate master structures and then used two-step replication process to regenerate. The fabricated fibrin scaffolds were highly porous and well interconnected. Culture of endothelial cells in the scaffolds resulted in directed lining and spreading of cells within a replicated pore network, whereas endothelial cells encapsulated in fibrin gel blocks showed chaotic and irregular distributions. These results demonstrated that the combination of 2PP and micromolding technique can supply complex 3D structures for tissue engineering.

Koroleva et al. [41] used 2PP to produce well-defined macroscopic scaffolds for engineering of neural tissue. Their scaffolds can be replicated by soft lithography, so production speed is relatively fast. Photo-cross-linkable poly(lactic acid) (PLA) was used to produce scaffolds by 2PP and soft lithography. PLA 3D scaffolds sustained a high degree (99%) of Schwann cell purity and provided a suitable substrate to support Schwann cell adhesion. Most of the Schwann cells in the scaffolds showed alignment of actin filaments and formation of focal contacts. These photo-cross-linked PLA scaffolds successfully support the growth of primary Schwann cells.

Claeyssens et al. [29] fabricated microstructures using 2PP process and the biodegradable copolymer poly(ε-caprolactone-co-trimethylenecarbonate)-b-poly(ethylene glycol)-b-poly(ε-caprolactone-co-trimethylene-carbonate) with 4,4′-bis(diethylamino)benzophenone as the photoinitiator. The minimum line width of structures was 4 μm, and the fabricated structure showed a fully interconnected 3D shape (Figure 2). Initial cytotoxicity was not detected, and cell proliferation speed was moderate. These proliferation results demonstrated that this material can be applied to the scaffold for tissue engineering.

Correa et al. [42] used 2PP to fabricate microstructures that contained chitosan, which is a biodegradable and biocompatible polymer that has applications in blood coagulation, soft tissue, and bone regeneration. Chitosan could provide microstructures with appealing properties for medical applications. The chitosan did not react chemically with the matrix resin and therefore retains its characteristics after the fabrication process.

Kufelt et al. [43] fabricated the 3D hydrogel microenvironments with predefined geometry and porosity using 2PP and chitosan. They explored a new synthesis of water-soluble photosensitive chitosan and the fabrication of well-defined microstructures from the generated materials. To modulate the mechanical and biochemical properties of the material, chitosan was combined and cross-linked with synthetic poly(ethylene glycol) diacrylate. For a biological adaption to the *in vivo* situation, chitosan was covalently cross-linked

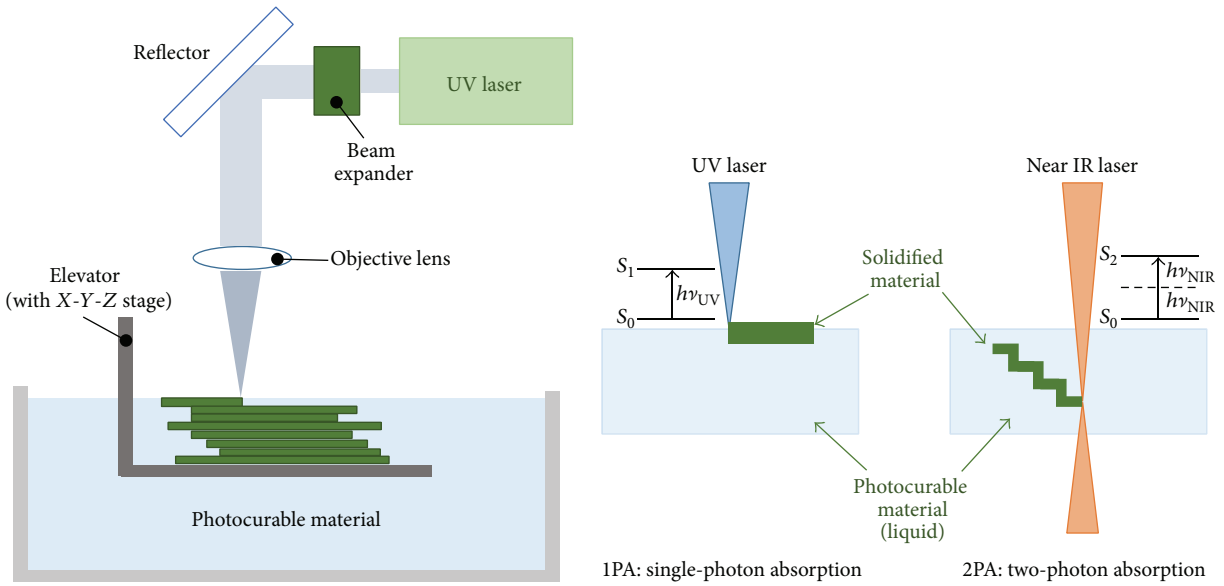

FIGURE 1: (a) Fundamental principle of MSTL technology. (b) Comparison between single-photon polymerization and two-photon polymerization.

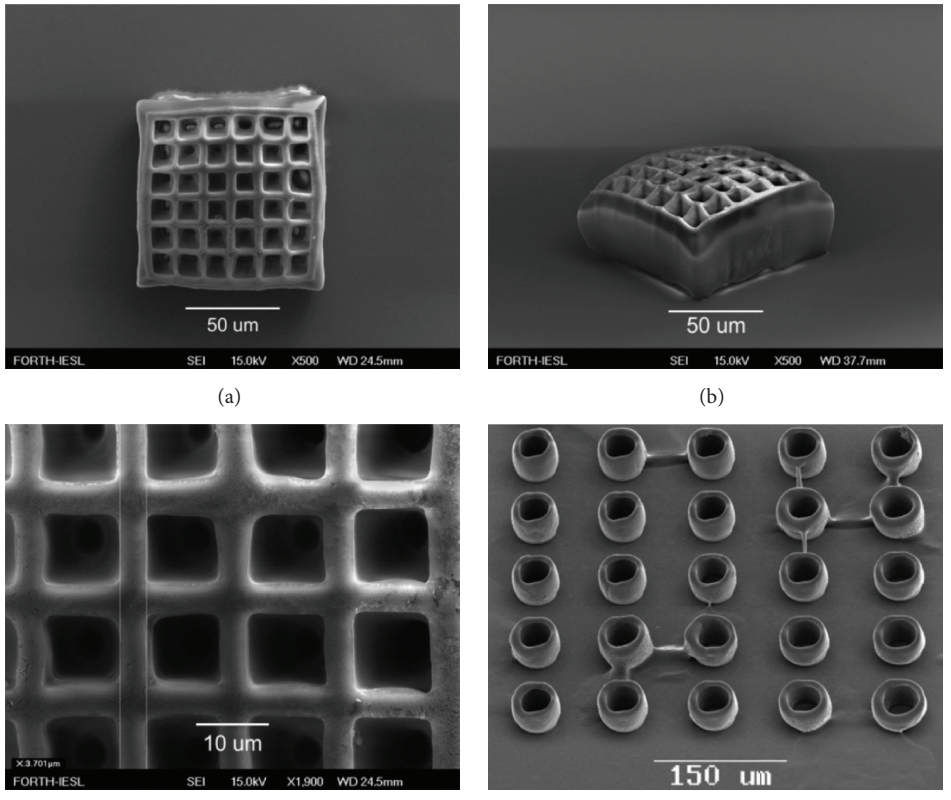

FIGURE 2: 3D structure fabricated using two-photon polymerization. (a) Top view, (b) side view, (c) detail, and (d) large cylindrical structures (figures were reproduced from [29] with permission of ACS Publications).

with a photosensitive modified vascular endothelial growth factor (VEGF). Performed *in vitro* studies revealed that modified chitosan is biocompatible and VEGF enhanced CH bioactivity. Furthermore, a 3D chitosan scaffold was successfully seeded with cells. From the study, the established chitosan showed a potential for future applications in tissue engineering.

Cha et al. [44] used 2PP to incorporate micropatterns on the scaffold. They fabricated 3D scaffolds with micropillar and microridge patterns on each layer and observed the effects of the patterns on cellular behaviors including adhesion, proliferation, and osteogenic differentiation. Preosteoblasts adhered significantly better to scaffolds with micropatterns than to a scaffold without a pattern. The expression results of osteogenic markers including ALP and Runx2 showed the superiority of scaffolds that had micropillar and microridge patterns. Thus, use of a femtosecond laser to print micropatterns on 3D scaffolds may be a useful method to encourage tissue regeneration.

Marino et al. [45] fabricated a trabecula-like structure, which was named "Osteoprint" that resembles the typical microenvironment of trabecular bone cells using two-photon polymerization process. Starting from microtomography images of the trabecular bone, they prepared several Osteoprints through two-photon polymerization and tested the behavior of SaOS-2 bone-like cells cultured on their structures. They found that Osteoprints deeply affect cellular behavior, determining an exit from the cell cycle and an enhancement of osteogenic differentiation. And they also found an upregulation of the genes involved in SaOS-2 cell maturation and an increase in hydroxyapatite production and accumulation upon SaOS-2 culture on the Osteoprints. Their finding showed the new perspectives in "bioinspired" approaches for tissue engineering and regenerative medicine.

Doraiswamy et al. [46] fabricated three-dimensional microstructured medical devices by 2PP of Ormocer organic–inorganic hybrid materials. Neuroblast-like cells and epithelial-like cells showed good viability of fabricated Ormocer. Microneedle arrays with unique geometries and Lego-like interlocking tissue engineering scaffolds were fabricated using 2PP. These results showed that 2PP can create biomedical microdevices with a larger range of sizes and shapes than can reactive ion etching, surface/bulk micromachining, injection molding, polysilicon micromolding, or other conventional microfabrication techniques.

In most biological studies, cell movement is a subject of ongoing study. Especially in cancer biology, the understanding of the cell migration is very important to estimate and forecast cancer metastasis. However, most related studies have been conducted in standard two-dimensional (2D) environments such as plastic plates coated with extracellular matrix, or glass tissue culture plates. Study of realistic cellular motion and migration requires an effective 3D biomimetic environment. Use of 2PP to fabricate such an environment with high resolution has been the subject of several studies.

Otuka et al. [47] used 2PP to fabricate microenvironment for *in situ* monitoring of cell growth and movement. They fabricated a microenvironment that was doped at specific site with ciprofloxacin (an antibiotic that is used in the treatment of diseases caused by *E. coli*) and that included microfences that can trap bacteria. Development of *E. coli* was inhibited near sites that were doped with ciprofloxacin, and the microfence traps increased the density of *E. coli* near them. These microenvironments showed potential as a platform for drug delivery system by promoting or inhibiting the growth of bacteria.

Zhang et al. [30] used poly(ethylene glycol) diacrylate (PEDGA) biomaterial to fabricate suspended web structures that exhibit positive or negative Poisson's ratio (NPR). The authors observed cellular responses involved in tuning Poisson's ratio in biological scaffolds and developed high-resolution NPR webs that demonstrated biaxial behavior during expansion or contraction as one or more cells applied local forces and moved the structures. The NPR structures fostered unusual cell division, and cells migrated toward regions that were stiffer than average (Figure 3). This 2PP process demonstrated that Poisson's ratio of photo-cross-linkable biomaterials can be tuned; this approach has potential applications in mechanobiology.

Raimondi et al. [48] applied femtosecond laser 2PP to fabricate 3D microscaffolds, or "niches," using a hybrid organic-inorganic photoresist. They developed two niche heights, 20 and 80–100 μm, and four lattice pore dimensions (10, 20, and 30 μm and graded) and they prepared primary rat mesenchymal stem cells (MSCs) to study cell viability, migration, and proliferation in the niches. MSCs preferentially stayed on/in the structures once they ran into them through random migration from the surrounding flat surface, invaded those with a lattice pore dimension greater than 10 μm, and adhered to the internal lattice while the cell nuclei acquired a roundish morphology. In the niches, the highest MSC density was found in those areas where proliferation was observed. The microgeometry inducing the highest cell density was 20 μm high with graded pores, in which cell invasion was favored in the central region of large porosity and cell adhesion was favored in the lateral regions of high scaffold surface density. Their result showed the crucial role played by the niche 3D geometry on MSC colonization in culture.

Jeon et al. [49] also used 2PP to fabricate patterns with various height and high aspect ratios (~10) and then used them in studies of cell guidance. They seeded fibroblasts on orthogonal mesh patterns (8 μm and 4 μm height, 5 μm and 5.5 μm height, and 5 μm and 6 μm height) and on parallel line patterns with different heights (1.5, 0.8, and 0.5 μm). The seeded fibroblasts received different contact strengths depending on the wall height. A threshold of approximately 1 μm in height influenced cell alignment both on mesh and on line patterns. This technology may be used in design of microdevices for controlling cell behavior and for investigating cell signal transduction.

Drug delivery is administration of pharmaceutical materials to achieve a therapeutic effect in living creatures. A drug delivery system (DDS) supplies a predetermined drug releasing profile which ensures an optimal absorption of the drug to improve its safety and efficacy. 3D microscale or nanoscale systems [50–55] made from various biomaterials may have applications [56–62] in DDSs, and 2PP has been evaluated as a tool for development of DDSs.

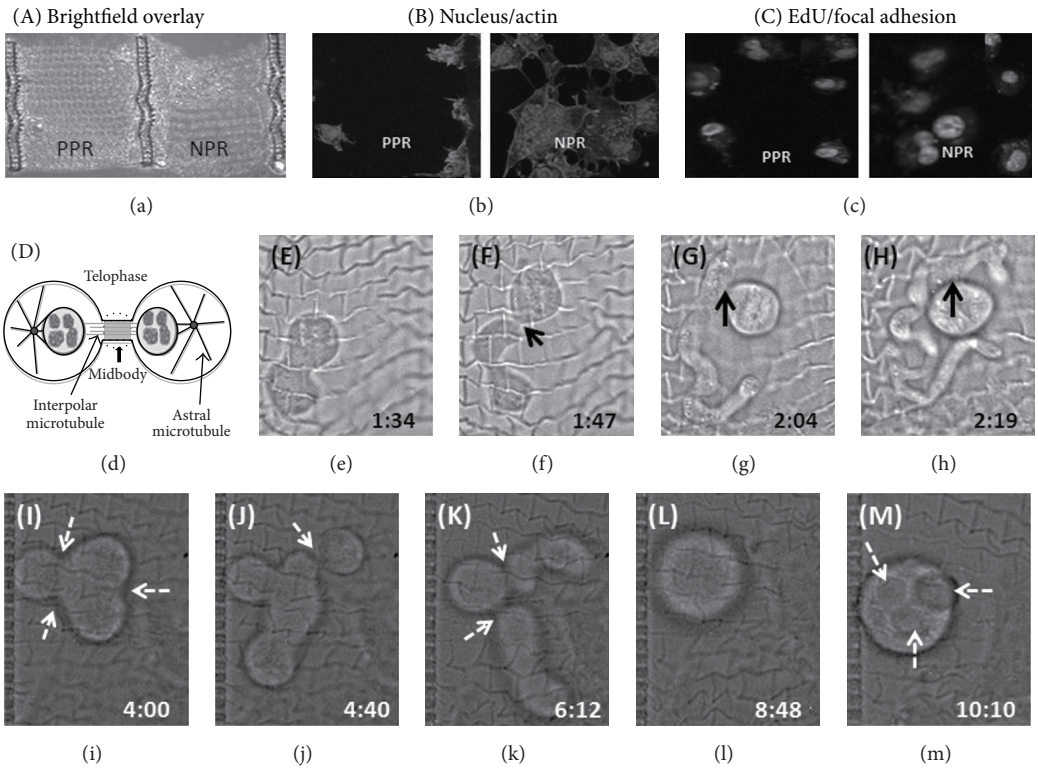

Figure 3: (a–c) Immunostaining of 10T1/2 cells for adhesion (nucleus, actin, and focal adhesions) and proliferation (EdU). (d) Schematic illustration showing cell in telophase: the cleavage furrow ingresses, compresses the midzone, and creates an intercellular bridge containing a microtubule midbody. In normal cell division, the bridge is resolved creating two daughter cells. The NPR structure induces aberrant cell-division response: (e–h) Abnormal cell division initiates and results in the formation of a long structure. Black arrow indicates persistent midbody connection. (i–m) Multiple sites of symmetric as well as asymmetric furrow formation during cell division on the NPR webs (dotted white arrows) (figures were reproduced from [30] with permission of John Wiley and Sons).

Chan et al. [63] used 2PP to deposit complex protein microstructures with submicrometer features and controllability. In bovine serum albumin (BSA) protein solution, the method produced 2D line voxels with lateral fabrication resolution of 200 nm and elliptical 3D spot voxels with dimensions of 400 nm (lateral) and 1.5 μm (axial). The authors fabricated BSA-based micropillar arrays and used them as platforms for cell niche studies. A study using fibroblasts showed good cell attachment and growth and good expression of adhesion molecules without the need for a matrix coating. This work presented a useful method to engineer protein microstructures with submicrometer topological features to mimic the native matrix niche. These structures have applications in cell-matrix interaction studies.

Turunen et al. [64] studied using picosecond and femtosecond lasers to induce photo-cross-linking of protein microstructures. The capability of a picosecond laser (Nd:YAG) to induce protein crosslinking by a multiphoton excitation was evaluated by fabricating 2D and 3D microstructures of bovine serum albumin (BSA), biotinylated bovine serum albumin (bBSA), and avidin. The authors fabricated sub-micrometer-scale and micrometer-scale structures from several different protein compositions and photosensitizers by varying the average laser power and scanning speed and then compared the surface topography and resolution of the resulting protein patterns to those of protein patterns fabricated using a femtosecond Ti:Sapphire laser. The study demonstrated that a low-cost Nd:YAG microlaser can be used for direct laser writing of protein microstructures.

Farsari et al. [65, 66] functionalized the surface of 3D structures fabricated using three-photon polymerization and then immobilized photosensitive biotin on the structure surface. The existence and distribution of biotin were measured using fluorescence microscopy and a surface acoustic sensor technique to detect the presence of avidin. The same research group has studied the immobilization of peptides. The methods developed by this group can be used to fabricate scaffolds for cell growth and tissue engineering.

Gittard et al. [67] fabricated microneedles with antimicrobial function for transdermal delivery of protein- and nucleic acid-based drugs. Existing microneedle-generated pores may allow microorganisms to penetrate the stratum corneum layer of the epidermis and infection. Therefore, the authors used 2PP, micromolding, and pulsed laser deposition to fabricate microneedles that had antimicrobial functionality. The authors fabricated needles from Ormocer and either coated them with silver or left them bare. The silver-coated Ormocer microneedles showed antibacterial properties but did not inhibit growth of human keratinocytes. This result showed that use of silver coating is an effective approach for

creating microneedles that have antimicrobial characteristics. In a follow-up study, the authors fabricated microneedle arrays that contain Polyethylene Glycol-Gentamicin Sulfate, which inhibited growth of *Staphylococcus aureus* bacteria [68].

2.1.2. Two-Photon Erosion Technology for Tissue Engineering. Photodegradation is the alteration of a molecule by infrared, visible, or ultraviolet radiation. Two-photon excitation using a pulsed laser induces degradation within hydrogels over multiple length scales by cleaving components within the cross-linked biomaterial. This method achieves submicrometer resolution and can erode the focal volume within the bulk material; these traits have been exploited to develop platforms for various *in vitro* studies.

Lee et al. [69] suggested a micropatterning technique that uses two-photon-induced erosion (2PIE) to control the 3D arrangement of biomolecules and cells at the micrometer scale. The authors fabricated a 3D micropattern of cell adhesive ligand (Arg-Gly-Asp-Ser: RGDS) in collagenase-sensitive poly(ethylene glycol-co-peptide) diacrylate hydrogels to guide cell migration along predefined 3D pathways and human dermal fibroblasts encapsulated within the micropatterned collagenase-sensitive hydrogels were located in the center of the hydrogel construct. After preparation of the 3D pathway, cells migrated along predefined 3D RGDS pathways. Their result showed the possibility of guiding tissue regeneration by using 3D scaffolds with highly defined microscale geometry.

Kloxin et al. [70] synthesized photodegradable poly(ethylene glycol) based hydrogel, which has physical and biological properties that can be modulated in the presence of cells by ultraviolet, visible, and two-photon irradiation. 3D channels that were fabricated using 2PIE within a hydrogel allowed migration of hydrogel-encapsulated cells, and variation of the gel composition induced chondrogenic differentiation of encapsulated stem cells. These photodegradable hydrogels showed promise as *in vitro* 3D cell culture platforms in which an interaction between cells and materials is elucidated by the processing information of cells. These methods may be useful in applications such as drug-delivery vehicles and tissue-engineering systems.

Tibbitt et al. [31] presented a PEG-based hydrogel in which the geometry and context of the extracellular environment were controlled by 2PIE (Figure 4). They characterized the 2PIE process and demonstrated its efficacy in cell culture. To erode a gel completely, they selected 2PIE parameters in the presence of cells and then eroded microscale structures on and in the gel to confirm the patterning resolution. They used 2PIE to erode the material at the cell-gel interface and remove cell adhesion sites selectively. Finally, they monitored the stem cell response by detachment between cells and soft materials. This technique allows users to manipulate precisely the context and geometry of a cell's underlying microenvironment.

2.1.3. Two-Photon Ablation Technology for Tissue Engineering. Two-photon lithography (2PL) can provide high-resolution material processing without requiring a chemical developer or a photomask [71, 72]. Intense pulses of femtosecond laser can cause nonlinear absorption processes (e.g., multiphoton-initiated avalanche ionization) that can damage transparent dielectrics [73]. However, heat exchange is limited during femtosecond pulsed laser irradiation, so thermal stress and collateral damage are minimized. Thus this laser can achieve submicrometer resolution when ablating biomaterials.

Jeon et al. [32] used a two-photon laser to write nanoscale chemical patterns on thin polymer film. Poly(ethylene glycol) methacrylate (PEG-MA) layers were prepared on quartz substrates, and then nonlinear absorbance of pairs of photons from femtosecond laser was used to ablate the underlying substrate. Single-shot ablation allowed the patterning of nanoscale features without a damage of the substrate (Figure 5). The diameter of the laser spot was 0.86 μm at $1/e^2$ width, and the exposed feature size on the substrate was ~80 nm in that condition. Fabricated patterns could control the adhesion and migration of 3T3 fibroblasts, so this study demonstrated the use of two-photon ablation technology to realize a microenvironment.

2.2. 3D Printing Based on Controlled Electrospinning. Electrospinning is a versatile 3D printing technique that uses a biopolymer; the method was first proposed in 1934 [26]. Electrospinning is based on the creation of fibers by ejecting an electrically charged viscoelastic polymer solution onto a collector. The travel pathway of the charged polymer solution is guided by a strong electric field that is generated by a high voltage between a polymer solution outlet and the collector guide [27]. By the control of solution conditions (pH, concentration, and solvent), device conditions (distance between tip and plate, strength of electric field, and dimensions of nozzle), and collection methods (plate versus rotating mandrel and speed of collection), this technique can produce ultrafine fibers with a wide range of diameters from several micrometers to a few nanometers. However, electrospun nanofibers undergo a whipping motion, so an electrospun product is normally a nonwoven mat of randomly oriented fibers. This characteristic has limited the use of this method to fabricate patient-customized architectures for use in tissue regeneration. However, various techniques to align and position the nanofibers have been developed, so the electrospinning technology has been utilized in tissue engineering and regenerative medicine area.

2.2.1. 2D Pattern Fabrication Using Controlled Electrospinning. Research on the morphology of nanofibrous structures has mainly focused on nanofiber alignment [33, 74–77]. However, although achieving alignment of the nanofiber was a significant breakthrough that allowed deposition of structured nanofiber mats, the precision of alignment is still limited by difficulties in controlling the geometric features of the electrospun mats and in introducing geometrical functionalities. Therefore, new methods, such as direct nanofiber patterning, a prepatterned conductive collector, have been studied.

Bellan and Craighead [78] used electric fields to confine and steer an electrospun polymer jet for controlled deposition of functional materials and used an electrode between the electrospinning tip and grounded sample to suppress the

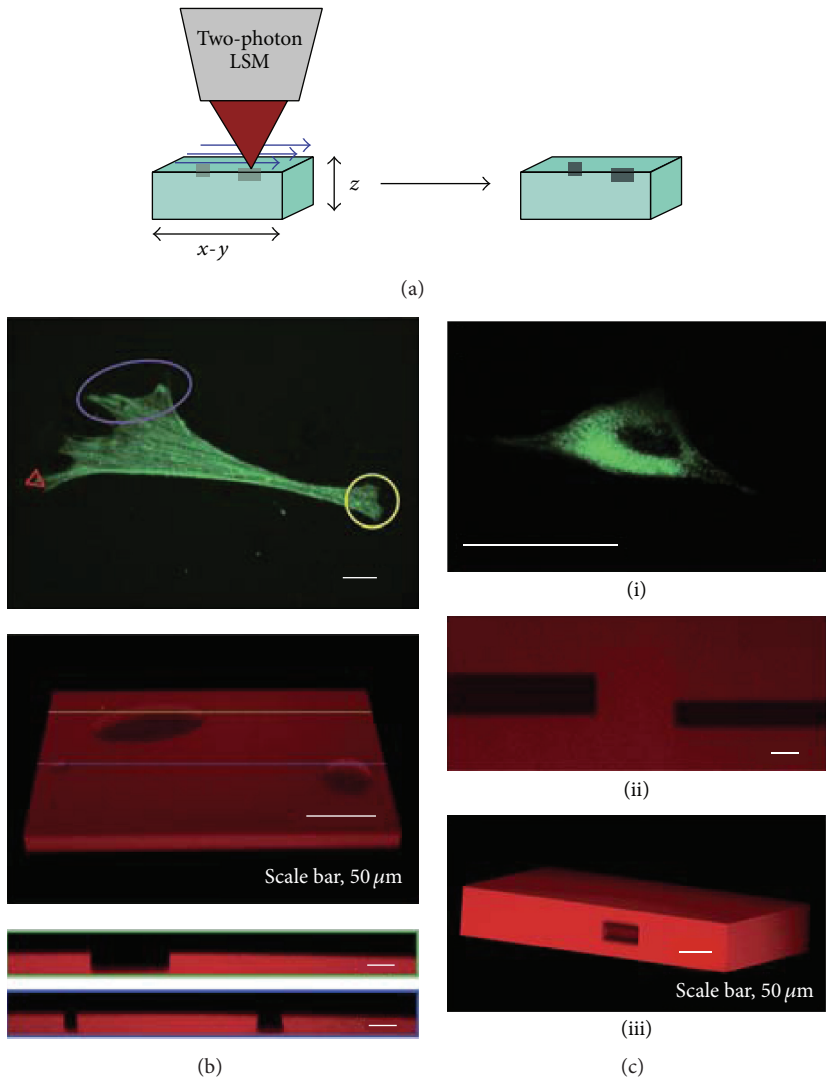

Figure 4: Feature formation to control ECM context and geometry. (a) Defined features can be patterned at the surface or within PEGdiPDA hydrogels by rastering the focal point of a two-photon laser scanning microscope (LSM, Zeiss LSM 710) through specific geometries using region of interest software. (b) Surface feature formation can be performed on size scales relevant to the cell (~1 to 100 mm) and spatially confined to desired regions to disrupt adhesion at the front or back side of adhered cells (purple oval and yellow circle) or to disrupt adhesion at individual filopodia (red triangle). To demonstrate this strategy, feature formation was performed in the absence of cells on the order of microns (red triangle) to 100 mm (purple oval) and was monitored with confocal microscopy (3D renderings of fluorescent confocal stacks and the corresponding cross sections, green and blue lines). (c) Features were also patterned within the bulk of PEGdiPDA hydrogels to motivate the utility of this approach for directing encapsulated cells ((c)(i)) to migrate down specific channels ((c)(ii)) or for defining the geometry of the cell niche ((c)(iii)). 20 mm and 30 mm wide channels were patterned into PEGdiPDA gels ((c)(ii)) for representative channel formation, and a 45 mm wide square cylinder was patterned into a gel ((c)(iii)) as a representative change to the geometry of the cell niche. Scale bars represent 20 mm, except as noted (figures were reproduced from [31] with permission of Royal Society of Chemistry).

chaotic whipping mode, thereby reducing the diameter of the characteristic spot. By modifying the electrode setup, they deposited isolated electrospun fibers in controlled positions and terminated electrospun fibers quickly. Their results will allow the increase in the complexity of the geometries that can be fabricated using electrospun nanofibers.

Dalton et al. [79] used melt electrospinning and demonstrated that simple nanofibrous patterns with line widths as small as 500 μm can be fabricated by increasing the tip-to-collector distance and reducing the speed of the plate collector. Electrospun fibers collected in focused spots were used in the patterning and drawing of a cell adhesive scaffold. Aligned electrospun fiber lines of 200–400 μm width could be applied continuously or discretely onto a slide mounted on an x-y stage. This direct electrospinning writing technique will provide scaffold-building devices suitable for tissue engineering applications.

Zhang and Chang [80] used electroconductive collectors to fabricate poly(lactic acid) (PLA) electrospun mats with different patterned structures (Figure 6). To control the

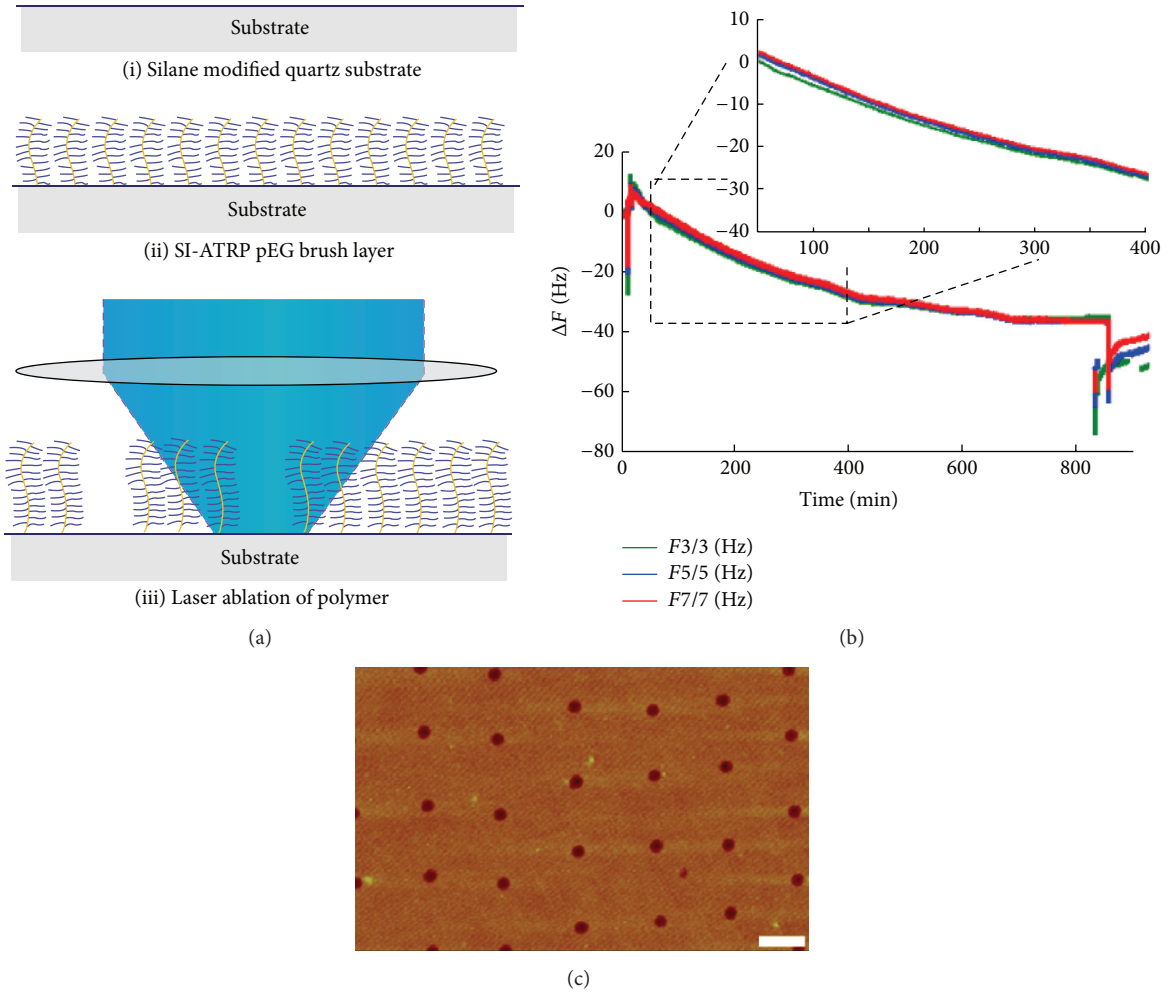

Figure 5: (a) Schematic of laser ablation. Polymer is grown from surface-bound ATRP initiator and ablated by a 100 fs, 400 nm laser pulse. (b) QCM-D measurement of polymer thickness. A measurement result indicates linear growth of the living radical polymerization, ensuring the smooth, uniform films necessary for consistent laser ablation. (c) AFM image of 250 nm dot pattern in 20 nm film (scale bar is 1 μm) (figures were reproduced from [32] with permission of ACS Publications).

patterns and architectures and the parameters that affect the formation of the patterns of the fibrous materials, the authors designed an electroconductive template. They demonstrated that protrusions on collectors are an important feature that may induce structures in the electrospun mat and that woven constructs can be fabricated by time-dependent control of the protrusion arrangement of the collector. These effects of protrusion arrangement and designed patterns have potential for use in supplying fibrous mats for biomedical applications.

Kharaziha et al. [81] fabricated elastomeric biodegradable poly(glycerol sebacate) (PGS):gelatin aligned nanofibrous scaffolds with various chemical composition, stiffness, and anisotropy. They incorporated PGS to create nanofibrous scaffolds that mimic the architecture of the left ventricular myocardium. They studied attachment, proliferation, alignment, and differentiation of neonatal rat cardiac fibroblast cells. They also studied protein expression and contractile function of cardiomyocytes on PGS:gelatin scaffolds. An aligned nanofibrous scaffold with 33 wt% PGS enhanced the cellular alignment of cardiomyocytes and elicited optimal synchronous contractions of them. These results suggest that the electrospun PGS:gelatin scaffold with an alignment had an important influence on the organization, phenotype, and contraction of cardiac cells and can be used in engineering of cardiac tissue.

2.2.2. 3D Scaffold Fabrication Using Controlled Electrospinning. Despite numerous benefits of electrospinning technology, it cannot easily fabricate macroscopically porous 3D nanofibrous scaffolds, due to their entangled fibers and densely packed membranous structure [82]. Although electrospun scaffolds provide favorable cellular interaction due to internal architectures, as in native tissue, cellular migration within 3D electrospun scaffolds has been limited because of their inherently small pore sizes. Furthermore, the porosity of these scaffolds cannot be controlled. To solve these problems,

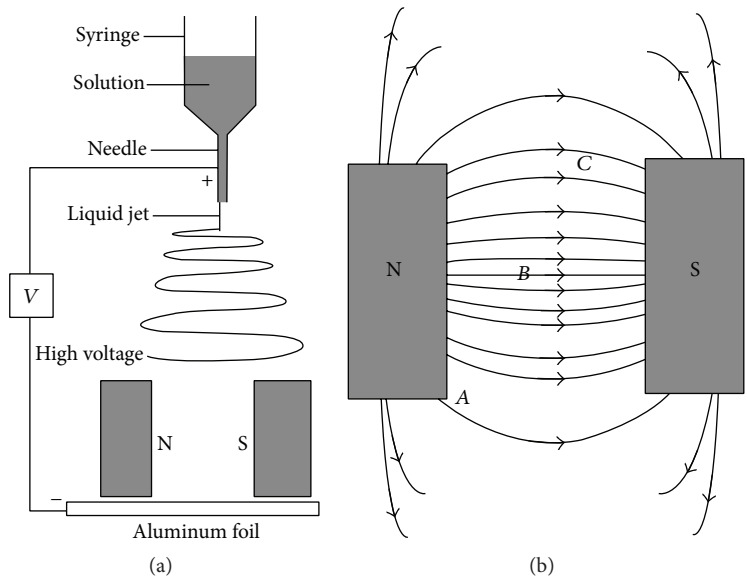

Figure 6: (a) Illustration of the apparatus for magnetic electrospinning (MES) to generate aligned fibers. The key component of the system is a magnetic field generated by two parallel-positioned permanent magnets. (b) Calculated magnetic field strength vectors in the region between the two magnets (top view) (figures were reproduced from [33] with permission of John Wiley and Sons).

some new approaches have been developed that control the electrode position to produce advanced electrospun 3D scaffolds.

Lee et al. [83] developed direct-write electrospinning (DWES) with improved focusing and scanning functionalities to generate nanofibrous mats. The authors demonstrated that DWES could control the geometry and dimensions of nanofibrous patterns and mats. Fabricated nanofibrous mats were used as patterns for cell alignment. 3D nanofibrous scaffolds with regular pores were developed by stacking the nanofibrous mats into a 3D structure [83, 84]. After preparing the 3D nanofibrous structures, they compared the cellular interactions induced by DWES, conventional electrospinning, and salt leaching technique. Cell migration to the inner space was better in the DWES scaffold than in the scaffold produced using conventional electrospinning. Scaffolds fabricated using DWES will eventually improve cellular migration into the core and aid in 3D tissue formation.

Teo et al. [33] controlled the motion of the electrospinning jet by use of knife edges to control the electrostatic field forces. The authors used polycaprolactone (PCL) as a biodegradable base material and fabricated tubular scaffolds with diagonally aligned fibers to be collected on a rotating tube. The tubular scaffold was formed with uniform thickness and possessed superior mechanical strength without any line of weakness. This technique may be used in development of strong tubular structures as blood vessel scaffolds.

Vaz et al. [85] used sequential multilayering electrospinning (ME) with a rotating mandrel-type collector to develop a scaffold that mimics both morphology and mechanical properties of a blood vessel. A bilayered tubular scaffold was composed of an outer layer composed of well-oriented stiff PLA fibers and an inner region composed of randomly oriented pliable PLA/PCL fibers. The degree of fiber orientation in the two layers was controlled by adjusting the rotation speed of the collector. Their scaffolds showed 10% elastic strain. They improved the attachment and proliferation of 3T3 mouse fibroblasts and human venous myofibroblasts. These results suggest that electrospun PLA/PCL bilayered tubular scaffolds with appropriate characteristics may be useful to guide regeneration of blood vessels.

Ignatova et al. [86] fabricated biocomponent nanofibrous mats by electrospinning mixed solutions of chitosan (Ch) or quaternized chitosan (Qch) and poly[(L-lactide)-co-(D,L-lactide)] (PLA). Cross-linked electrospun Ch/PLA and QCh/PLA mats inhibited growth of the *S. aureus* and *E. coli* more effectively than did solvent-cast film fabricated using the same materials. The reason for the difference was that Ch and QCh that were incorporated into electrospun mats decreased the ability of the bacteria to adhere to them. These hybrid nanofibrous mats may be useful in wound-healing applications.

Kim and Park [87] fabricated biodegradable polymeric nanocylinders by degradation of electrospun nanofibers. To make nanocylinders, nanofiber aggregates were uniformly dispersed in aqueous solution by aminolytic degradation of long electrospun fibers for reassembly of the fibers with controllable orientation and architecture. From transverse fragmentation of semicrystalline poly(L-lactic acid) (PLA) nanofibers, cylindrical and biodegradable nanomaterials with various aspect ratios were prepared. This approach showed the fabrication of ECM-mimicking nanofilaments which could potentially be assembled into highly ordered structures.

Zhang and Chang [34] suggested a static method to fabricate 3D fibrous tubes composed of ultrafine electrospun fibers (Figure 7). They used a 3D collecting template based on manipulation of electric fields and forces to fabricate 3D architecture. This technique can fabricate micro- and macrotubes with multiple micropatterns, multiple interconnected

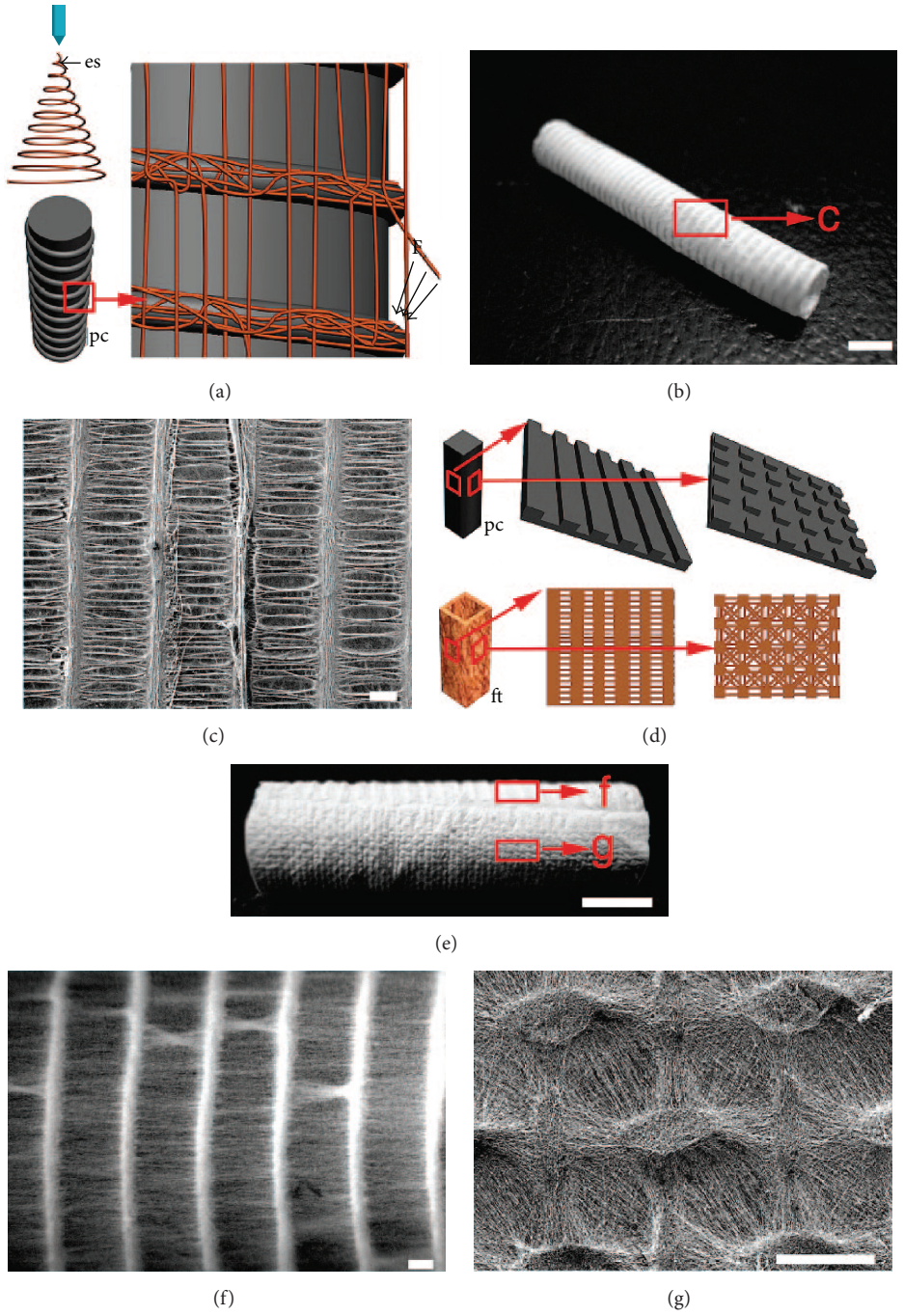

FIGURE 7: (a) Schematic illustration of collecting process using a cylindrical collector with equally spaced circular protrusions (es, electrospinning process; pc, patterned collector). (b) A fibrous tube with patterned architectures (scale bar = 5 mm). (c) Magnified image of panel (b) (scale bar = 200 μm). (d) Schematic illustration of collectors with two different patterns and relevant fibrous tube (pc, patterned collector; ft, fibrous tube). (e) A fibrous tube with two different patterns (scale bar = 5 mm). (f, g) Magnified images of two different patterns of panel (e) (scale bar = 200 μm) (figures were reproduced from [34] with permission of ACS Publications).

tubes, and many tubes with the same or different sizes, structure, shapes, and patterns. The authors investigated parameters that can affect the order degree of patterns. This technique to control the patterned architecture has many biomedical and industrial applications.

Aligned nanofibrous scaffolds fabricated using electrospinning can affect cell and matrix organization. However, their widespread application has been impeded by the poor cell infiltration due to the tight packing of the fibers. Therefore, Baker et al. [88] suggested tunable composite

nanofibrous scaffolds with water-soluble sacrificial fibers. Composites were composed of two fibers: slow-degrading poly(ε-caprolactone) (PCL) and water-soluble poly(ethylene oxide) (PEO), which is removed to increase the internal pore size in the fabricated 3D scaffold. PEO was spontaneously degraded by hydration, thereby leaving large pores that facilitated cellular infiltration. Although more than half of the initial fibers were removed, the remaining scaffold induced sufficient cell alignment and formation of a highly organized extracellular matrix at several length scales, and 3D cartilage tissue (>1 mm thick) was formed at 12 weeks after implantation. This technique can be used to support regeneration of load-bearing fibrous tissues.

3. Summary

This review has described 3D printing technologies with nanometer resolution for use in tissue engineering. 3D printing technologies have great advantages over traditional scaffold fabrication methods in the control of porosity, pore size, and interconnectivity. 3D printing methods can fabricate a 3D scaffold as designed, so they can be used to standardize 3D scaffolds. Among various 3D printing methods, two-photon laser-based nanofabrication and controlled electrospinning have received attention in various areas of tissue engineering due to their abilities to fabricate structures with high surface-to-volume ratio and highly interconnected porous architecture at submicrometer resolution. Use of these methods has yielded precise 3D scaffolds that mimic the organization of the extracellular matrix, and their characteristics have helped to study unknown cellular behaviors including adhesion, proliferation, and differentiation. The scaffolds have been used in studies of molecular biology and cell dynamics, so new possibilities for the improvement of tissue regeneration have been suggested. Future development of 3D nanoscaffolds should focus on increasing the precision of scaffold fabrication systems, on identifying new biomaterials, and on the role of biomolecules in cell behaviors such as adhesion, proliferation, and differentiation. Given the concentration on these topics, 3D nanoprinting technologies will become important tools in tissue engineering in the near future. High-resolution 3D biomimetic environments will become useful substrates in the search for mechanisms of vital phenomenon.

Conflict of Interests

There is no potential conflict of interests to disclose for this work.

Acknowledgments

This research was supported by Gachon University Gil Medical Center (Grant no. FRD2014-02) and the National Research Foundation of Korea (NRF) grant funded by the Korea government (no. 2014R1A1A2A16054777).

References

[1] R. Thomson, M. Yaszemski, and A. G. Mikos, "Polymer scaffold processing," in *Principles of Tissue Engineering*, R. Lanza, R. Langer, and W. Chick, Eds., pp. 263–272, R.G. Landes Company, Austin, Tex, USA, 1997.

[2] C. M. Agrawal, K. A. Athanasiou, and J. D. Heckman, "Biodegradable PLA-PGA polymers for tissue engineering in orthopaedics," *Materials Science Forum*, vol. 250, pp. 115–128, 1997.

[3] R. B. Langer, "Selected advances in drug delivery and tissue engineering," *Journal of Controlled Release*, vol. 62, no. 1-2, pp. 7–11, 1999.

[4] L. Lu and A. G. Mikos, "The importance of new processing techniques in tissue engineering," *MRS Bulletin*, vol. 21, no. 11, pp. 28–32, 1996.

[5] T. Ozdemir, A. M. Higgins, and J. L. Brown, "Osteoinductive biomaterial geometries for bone regenerative engineering," *Current Pharmaceutical Design*, vol. 19, no. 19, pp. 3446–3455, 2013.

[6] W. Chen, Y. Tabata, and Y. W. Tong, "Fabricating tissue engineering scaffolds for simultaneous cell growth and drug delivery," *Current Pharmaceutical Design*, vol. 16, no. 21, pp. 2388–2394, 2010.

[7] A. G. Mikos, A. J. Thorsen, L. A. Czerwonka et al., "Preparation and characterization of poly(L-lactic acid) foams," *Polymer*, vol. 35, no. 5, pp. 1068–1077, 1994.

[8] A. G. Mikos, Y. Bao, L. G. Cima, D. E. Ingber, J. P. Vacanti, and R. B. Langer, "Preparation of poly(glycolic acid) bonded fiber structures for cell attachment and transplantation," *Journal of Biomedical Materials Research*, vol. 27, no. 2, pp. 183–189, 1993.

[9] H. Hall, "Modified fibrin hydrogel matrices: both, 3D-scaffolds and local and controlled release systems to stimulate angiogenesis," *Current Pharmaceutical Design*, vol. 13, no. 35, pp. 3597–3607, 2007.

[10] G. M. Harris, K. Rutledge, Q. Cheng, J. Blanchette, and E. Jabbarzadeh, "Strategies to direct angiogenesis within scaffolds for bone tissue engineering," *Current Pharmaceutical Design*, vol. 19, no. 19, pp. 3456–3465, 2013.

[11] E. Vanderleyden, S. Mullens, J. Luyten, and P. Dubruel, "Implantable (bio)polymer coated titanium scaffolds: a review," *Current Pharmaceutical Design*, vol. 18, no. 18, pp. 2576–2590, 2012.

[12] A. G. Mikos, G. Sarakinos, S. M. Leite, J. P. Vacant, and R. Langer, "Laminated three-dimensional biodegradable foams for use in tissue engineering," *Biomaterials*, vol. 14, no. 5, pp. 323–330, 1993.

[13] D. J. Mooney, D. F. Baldwin, N. P. Suh, J. P. Vacanti, and R. Langer, "Novel approach to fabricate porous sponges of poly(D,L-lactic-co-glycolic acid) without the use of organic solvents," *Biomaterials*, vol. 17, no. 14, pp. 1417–1422, 1996.

[14] I. Zein, D. W. Hutmacher, K. C. Tan, and S. H. Teoh, "Fused deposition modeling of novel scaffold architectures for tissue engineering applications," *Biomaterials*, vol. 23, no. 4, pp. 1169–1185, 2002.

[15] T. D. Roy, J. L. Simon, J. L. Ricci, E. D. Rekow, V. P. Thompson, and J. R. Parsons, "Performance of hydroxyapatite bone repair scaffolds created via three-dimensional fabrication techniques," *Journal of Biomedical Materials Research—Part A*, vol. 67, no. 4, pp. 1228–1237, 2003.

[16] T.-M. G. Chu, D. G. Orton, S. J. Hollister, S. E. Feinberg, and J. W. Halloran, "Mechanical and in vivo performance of hydroxyapatite implants with controlled architectures," *Biomaterials*, vol. 23, no. 5, pp. 1283–1293, 2002.

[17] F. Pati, J. Jang, D.-H. Ha et al., "Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink," *Nature Communications*, vol. 5, article 3935, 2014.

[18] R. A. Barry III, R. F. Shepherd, J. N. Hanson, R. G. Nuzzo, P. Wiltzius, and J. A. Lewis, "Direct-write assembly of 3D hydrogel scaffolds for guided cell growth," *Advanced Materials*, vol. 21, no. 23, pp. 2407–2410, 2009.

[19] M. N. Cooke, J. P. Fisher, D. Dean, C. Rimnac, and A. G. Mikos, "Use of stereolithography to manufacture critical-sized 3D biodegradable scaffolds for bone ingrowth," *Journal of Biomedical Materials Research—Part B: Applied Biomaterials*, vol. 64, no. 2, pp. 65–69, 2003.

[20] J. W. Lee, P. X. Lan, B. Kim, G. Lim, and D.-W. Cho, "Fabrication and characteristic analysis of a poly(propylene fumarate) scaffold using micro-stereolithography technology," *Journal of Biomedical Materials Research—Part B: Applied Biomaterials*, vol. 87, no. 1, pp. 1–9, 2008.

[21] W.-S. Chu, S.-Y. Jeong, J. K. Pandey, S.-H. Ahn, J.-H. Lee, and S.-C. Chi, "Fabrication of composite drug delivery system using nano composite deposition system and in vivo characterization," *International Journal of Precision Engineering and Manufacturing*, vol. 9, no. 2, pp. 81–83, 2008.

[22] K. W. Lee, S. Wang, B. C. Fox, E. L. Ritman, M. J. Yaszemski, and L. Lu, "Poly(propylene fumarate) bone tissue engineering scaffold fabrication using stereolithography: effects of resin formulations and laser parameters," *Biomacromolecules*, vol. 8, no. 4, pp. 1077–1084, 2007.

[23] P. X. Lan, J. W. Lee, Y.-J. Seol, and D.-W. Cho, "Development of 3D PPF/DEF scaffolds using micro-stereolithography and surface modification," *Journal of Materials Science: Materials in Medicine*, vol. 20, no. 1, pp. 271–279, 2009.

[24] J.-H. Shim, J. Y. Kim, J. K. Park et al., "Effect of thermal degradation of SFF-based PLGA Scaffolds fabricated using a multi-head deposition system followed by change of cell growth rate," *Journal of Biomaterials Science, Polymer Edition*, vol. 21, no. 8-9, pp. 1069–1080, 2010.

[25] Y.-M. Ha, I.-B. Park, H.-C. Kim, and S.-H. Lee, "Three-dimensional microstructure using partitioned cross-sections in projection microstereolithography," *International Journal of Precision Engineering and Manufacturing*, vol. 11, no. 2, pp. 335–340, 2010.

[26] Z.-M. Huang, Y.-Z. Zhang, M. Kotaki, and S. Ramakrishna, "A review on polymer nanofibers by electrospinning and their applications in nanocomposites," *Composites Science and Technology*, vol. 63, no. 15, pp. 2223–2253, 2003.

[27] E. D. Boland, J. A. Matthews, K. J. Pawlowski, D. G. Simpson, G. E. Wnek, and G. L. Bowlin, "Electrospinning collagen and elastin: preliminary vascular tissue engineering," *Frontiers in Bioscience*, vol. 9, pp. 1422–1432, 2004.

[28] W. Liu, Y. Li, J. Liu, X. Niu, Y. Wang, and D. Li, "Application and performance of 3D printing in nanobiomaterials," *Journal of Nanomaterials*, vol. 2013, Article ID 681050, 7 pages, 2013.

[29] F. Claeyssens, E. A. Hasan, A. Gaidukeviciute et al., "Three-dimensional biodegradable structures fabricated by two-photon polymerization," *Langmuir*, vol. 25, no. 5, pp. 3219–3223, 2009.

[30] W. Zhang, P. Soman, K. Meggs, X. Qu, and S. Chen, "Tuning the poisson's ratio of biomaterials for investigating cellular response," *Advanced Functional Materials*, vol. 23, no. 25, pp. 3226–3232, 2013.

[31] M. W. Tibbitt, A. M. Kloxin, K. U. Dyamenahalli, and K. S. Anseth, "Controlled two-photon photodegradation of PEG hydrogels to study and manipulate subcellular interactions on soft materials," *Soft Matter*, vol. 6, no. 20, pp. 5100–5108, 2010.

[32] H. Jeon, R. Schmidt, J. E. Barton et al., "Chemical patterning of ultrathin polymer films by direct-write multiphoton lithography," *Journal of the American Chemical Society*, vol. 133, no. 16, pp. 6138–6141, 2011.

[33] W. E. Teo, M. Kotaki, X. M. Mo, and S. Ramakrishna, "Porous tubular structures with controlled fibre orientation using a modified electrospinning method," *Nanotechnology*, vol. 16, no. 6, pp. 918–924, 2005.

[34] D. Zhang and J. Chang, "Electrospinning of three-dimensional nanofibrous tubes with controllable architectures," *Nano Letters*, vol. 8, no. 10, pp. 3283–3287, 2008.

[35] H. Kodama, "Automatic method for fabricating a three-dimensional plastic model with photo-hardening polymer," *Review of Scientific Instruments*, vol. 52, no. 11, pp. 1770–1773, 1981.

[36] T. Nakai and Y. Marutani, "Fabrication of 3-D prototypes by using ultraviolet laser and liquid photopolymer," in *Proceedings of the Conference on Lasers and Electro-Optics*, ME-2, San Francisco, Calif, USA, June 1986.

[37] H.-W. Kang, I. H. Lee, and D.-W. Cho, "Development of an assembly-free process based on virtual environment for fabricating 3D microfluidic systems using microstereolithography technology," *Journal of Manufacturing Science and Engineering*, vol. 126, no. 4, pp. 766–771, 2004.

[38] R. Liska, M. Schuster, R. Inführ et al., "Photopolymers for rapid prototyping," *Journal of Coatings Technology Research*, vol. 4, no. 4, pp. 505–510, 2007.

[39] J.-F. Xing, X.-Z. Dong, W.-Q. Chen et al., "Improving spatial resolution of two-photon microfabrication by using photoinitiator with high initiating efficiency," *Applied Physics Letters*, vol. 90, no. 13, Article ID 131106, 2007.

[40] A. Koroleva, S. Gittard, S. Schlie, A. Deiwick, S. Jockenhoevel, and B. Chichkov, "Fabrication of fibrin scaffolds with controlled microscale architecture by a two-photon polymerization-micromolding technique," *Biofabrication*, vol. 4, no. 1, Article ID 015001, 2012.

[41] A. Koroleva, A. A. Gill, I. Ortega et al., "Two-photon polymerization-generated and micromolding-replicated 3D scaffolds for peripheral neural tissue engineering applications," *Biofabrication*, vol. 4, no. 2, Article ID 025005, 2012.

[42] D. S. Correa, P. Tayalia, G. Cosendey et al., "Two-photon polymerization for fabricating structures containing the biopolymer chitosan," *Journal of Nanoscience and Nanotechnology*, vol. 9, no. 10, pp. 5845–5849, 2009.

[43] O. Kufelt, A. El-Tamer, C. Sehring, M. Meißner, S. Schlie-Wolter, and B. N. Chichkov, "Water-soluble photopolymerizable chitosan hydrogels for biofabrication via two-photon polymerization," *Acta Biomaterialia*, vol. 18, pp. 186–195, 2015.

[44] H. D. Cha, J. M. Hong, T.-Y. Kang, J. W. Jung, D.-H. Ha, and D.-W. Cho, "Effects of micro-patterns in three-dimensional scaffolds for tissue engineering applications," *Journal of Micromechanics and Microengineering*, vol. 22, no. 12, Article ID 125002, 2012.

[45] A. Marino, C. Filippeschi, G. G. Genchi, V. Mattoli, B. Mazzolai, and G. Ciofani, "The Osteoprint: a bioinspired two-photon polymerized 3-D structure for the enhancement of bone-like cell differentiation," *Acta Biomaterialia*, vol. 10, no. 10, pp. 4304–4313, 2014.

[46] A. Doraiswamy, C. Jin, R. J. Narayan et al., "Two-photon induced polymerization of organic-inorganic hybrid biomaterials for microstructured medical devices," *Acta Biomaterialia*, vol. 2, no. 3, pp. 267–275, 2006.

[47] A. J. G. Otuka, D. S. Corrêa, C. R. Fontana, and C. R. Mendonça, "Direct laser writing by two-photon polymerization as a tool for developing microenvironments for evaluation of bacterial growth," *Materials Science and Engineering C*, vol. 35, no. 1, pp. 185–189, 2014.

[48] M. T. Raimondi, S. M. Eaton, M. Laganà et al., "Three-dimensional structural niches engineered via two-photon laser polymerization promote stem cell homing," *Acta Biomaterialia*, vol. 9, no. 1, pp. 4579–4584, 2013.

[49] H. Jeon, H. Hidai, D. J. Hwang, and C. P. J. Grigoropoulos, "Fabrication of arbitrary polymer patterns for cell study by two-photon polymerization process," *Journal of Biomedical Materials Research—Part A*, vol. 93, no. 1, pp. 56–66, 2010.

[50] L. L. Lebel, B. Aissa, M. A. El Khakani, and D. Therriault, "Ultraviolet-assisted direct-write fabrication of carbon nanotube/polymer nanocomposite microcoils," *Advanced Materials*, vol. 22, no. 5, pp. 592–596, 2010.

[51] T. D. Brown, P. D. Dalton, and D. W. Hutmacher, "Direct writing by way of melt electrospinning," *Advanced Materials*, vol. 23, no. 47, pp. 5651–5657, 2011.

[52] V. Chan, P. Zorlutuna, J. H. Jeong, H. Kong, and R. Bashir, "Three-dimensional photopatterning of hydrogels using stereolithography for long-term cell encapsulation," *Lab on a Chip*, vol. 10, no. 16, pp. 2062–2070, 2010.

[53] J. Zheng, H. Xie, W. Yu et al., "Enhancement of surface graft density of MPEG on alginate/chitosan hydrogel microcapsules for protein repellency," *Langmuir*, vol. 28, no. 37, pp. 13261–13273, 2012.

[54] U. Gbureck, E. Vorndran, F. A. Müller, and J. E. Barralet, "Low temperature direct 3D printed bioceramics and biocomposites as drug release matrices," *Journal of Controlled Release*, vol. 122, no. 2, pp. 173–180, 2007.

[55] G. M. Gratson, F. García-Santamaría, V. Lousse et al., "Direct-write assembly of three-dimensional photonic crystals: conversion of polymer scaffolds to silicon hollow-woodpile structures," *Advanced Materials*, vol. 18, no. 4, pp. 461–465, 2006.

[56] W. Wu, A. Deconinck, and J. A. Lewis, "Omnidirectional printing of 3D microvascular networks," *Advanced Materials*, vol. 23, no. 24, pp. 178–183, 2011.

[57] V. Karageorgiou and D. Kaplan, "Porosity of 3D biomaterial scaffolds and osteogenesis," *Biomaterials*, vol. 26, no. 27, pp. 5474–5491, 2005.

[58] A. Gould, Y. Ji, T. L. Aboye, and J. A. Camarero, "Cyclotides, a novel ultrastable polypeptide scaffold for drug discovery," *Current Pharmaceutical Design*, vol. 17, no. 38, pp. 4294–4307, 2011.

[59] S. Maya, B. Sarmento, A. Nair, N. S. Rejinold, S. V. Nair, and R. Jayakumar, "Smart stimuli sensitive nanogels in cancer drug delivery and imaging: a review," *Current Pharmaceutical Design*, vol. 19, no. 41, pp. 7203–7218, 2013.

[60] G. G. Adams and S. E. Harding, "Drug delivery systems for the treatment of diabetes mellitus: state of the art," *Current Pharmaceutical Design*, vol. 19, no. 41, pp. 7244–7263, 2013.

[61] C. W. Gan, S. Chien, and S.-S. Feng, "Nanomedicine: enhancement of chemotherapeutical efficacy of docetaxel by using a biodegradable nanoparticle formulation," *Current Pharmaceutical Design*, vol. 16, no. 21, pp. 2308–2320, 2010.

[62] J. Xie, C. Lei, Y. Hu, G. K. Gay, N. H. B. Jamali, and C.-H. Wang, "Nanoparticulate formulations for paclitaxel delivery across MDCK cell monolayer," *Current Pharmaceutical Design*, vol. 16, no. 21, pp. 2331–2340, 2010.

[63] B. P. Chan, J. N. Ma, J. Y. Xu, C. W. Li, J. P. Cheng, and S. H. Cheng, "Femto-second Laser-based free writing of 3D protein microstructures and micropatterns with Sub-micrometer features: a study on voxels, porosity, and cytocompatibility," *Advanced Functional Materials*, vol. 24, no. 3, pp. 277–294, 2014.

[64] S. Turunen, E. Käpylä, K. Terzaki et al., "Pico- and femtosecond laser-induced crosslinking of protein microstructures: evaluation of processability and bioactivity," *Biofabrication*, vol. 3, no. 4, Article ID 045002, 2011.

[65] T. S. Drakakis, G. Papadakis, K. Sambani et al., "Construction of three-dimensional biomolecule structures employing femtosecond lasers," *Applied Physics Letters*, vol. 89, no. 14, Article ID 144108, 2006.

[66] M. Farsari, M. Vamvakaki, and B. N. Chichkov, "Multiphoton polymerization of hybrid materials," *Journal of Optics*, vol. 12, no. 12, Article ID 124001, 2010.

[67] S. D. Gittard, R. J. Narayan, C. Jin et al., "Pulsed laser deposition of antimicrobial silver coating on Ormocer microneedles," *Biofabrication*, vol. 1, no. 4, Article ID 041001, 2009.

[68] S. D. Gittard, A. Ovsianikov, H. Akar et al., "Two photon polymerization-micromolding of polyethylene glycol-gentamicin sulfate microneedles," *Advanced Engineering Materials*, vol. 12, no. 4, pp. B77–B82, 2010.

[69] S.-H. Lee, J. J. Moon, and J. L. West, "Three-dimensional micropatterning of bioactive hydrogels via two-photon laser scanning photolithography for guided 3D cell migration," *Biomaterials*, vol. 29, no. 20, pp. 2962–2968, 2008.

[70] A. M. Kloxin, A. M. Kasko, C. N. Salinas, and K. S. Anseth, "Photodegradable hydrogels for dynamic tuning of physical and chemical properties," *Science*, vol. 324, no. 5923, pp. 59–63, 2009.

[71] D. A. Higgins, T. A. Everett, A. Xie, S. M. Forman, and T. Ito, "High-resolution direct-write multiphoton photolithography in poly(methylmethacrylate) films," *Applied Physics Letters*, vol. 88, no. 18, Article ID 184101, 2006.

[72] S. Ibrahim, D. A. Higgins, and L. T. Ito, "Direct-write multiphoton photolithography: a systematic study of the etching behaviors in various commercial polymers," *Langmuir*, vol. 23, no. 24, pp. 12406–12412, 2007.

[73] C. P. Grigoropoulos, *Transport in Laser Microfabrication: Fundamentals and Applications*, Cambridge University Press, New York, NY, USA, 2009.

[74] P. Katta, M. Alessandro, R. D. Ramsier, and G. G. Chase, "Continuous electrospinning of aligned polymer nanofibers onto a wire drum collector," *Nano Letters*, vol. 4, no. 11, pp. 2215–2218, 2004.

[75] D. Li, Y. Wang, and Y. Xia, "Electrospinning nanofibers as uniaxially aligned arrays and layer-by-layer stacked films," *Advanced Materials*, vol. 16, no. 4, pp. 361–366, 2004.

[76] B. Sundaray, V. Subramanian, T. S. Natarajan, R.-Z. Xiang, C.-C. Chang, and W.-S. Fann, "Electrospinning of continuous aligned polymer fibers," *Applied Physics Letters*, vol. 84, no. 7, article 1222, 2004.

[77] D. Yang, B. Lu, Y. Zhao, and X. Jiang, "Fabrication of aligned fibrous arrays by magnetic electrospinning," *Advanced Materials*, vol. 19, no. 21, pp. 3702–3706, 2007.

[78] L. M. Bellan and H. G. Craighead, "Control of an electrospinning jet using electric focusing and jet-steering fields," *Journal of Vacuum Science and Technology B*, vol. 24, no. 6, pp. 3179–3183, 2006.

[79] P. D. Dalton, N. T. Joergensen, J. Groll, and M. Moeller, "Patterned melt electrospun substrates for tissue engineering," *Biomedical Materials*, vol. 3, no. 3, Article ID 034109, 2008.

[80] D. Zhang and J. Chang, "Patterning of electrospun fibers using electroconductive templates," *Advanced Materials*, vol. 19, no. 21, pp. 3664–3667, 2007.

[81] M. Kharaziha, M. Nikkhah, S.-R. Shin et al., "PGS:gelatin nanofibrous scaffolds with tunable mechanical and structural properties for engineering cardiac tissues," *Biomaterials*, vol. 34, no. 27, pp. 6355–6366, 2013.

[82] T. G. Kim, H. Shin, and D. W. Lim, "Biomimetic scaffolds for tissue engineering," *Advanced Functional Materials*, vol. 22, no. 12, pp. 2446–2468, 2012.

[83] J. Lee, S. Y. Lee, J. Jang, Y. H. Jeong, and D.-W. Cho, "Fabrication of patterned nanofibrous mats using direct-write electrospinning," *Langmuir*, vol. 28, no. 18, pp. 7267–7275, 2012.

[84] J. Lee, J. Jang, H. Oh, Y. H. Jeong, and D.-W. Cho, "Fabrication of a three-dimensional nanofibrous scaffold with lattice pores using direct-write electrospinning," *Materials Letters*, vol. 93, pp. 397–400, 2013.

[85] C. M. Vaz, S. van Tuijl, C. V. C. Bouten, and F. P. T. Baaijens, "Design of scaffolds for blood vessel tissue engineering using a multi-layering electrospinning technique," *Acta Biomaterialia*, vol. 1, no. 5, pp. 575–582, 2005.

[86] M. Ignatova, N. Manolova, N. Markova, and I. Rashkov, "Electrospun non-woven nanofibrous hybrid mats based on chitosan and PLA for wound-dressing applications," *Macromolecular Bioscience*, vol. 9, no. 1, pp. 102–111, 2009.

[87] T. G. Kim and T. G. Park, "Biodegradable polymer nanocylinders fabricated by transverse fragmentation of electrospun nanofibers through aminolysis," *Macromolecular Rapid Communications*, vol. 29, no. 14, pp. 1231–1236, 2008.

[88] B. M. Baker, R. P. Shah, A. M. Silverstein, J. L. Esterhai, J. A. Burdick, and R. L. Mauck, "Sacrificial nanofibrous composites provide instruction without impediment and enable functional tissue formation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 35, pp. 14176–14181, 2012.