Solid freeform fabrication technology applied to tissue engineering with various biomaterials

Young-Joon Seol,†a Tae-Yun Kanga and Dong-Woo Choab

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An important component in tissue engineering is the three-dimensional (3D) scaffold, which guides cells to form target tissue, maintains tissue volume, and provides sufficient structural support during tissue regeneration. However, until recently, conventional scaffold fabrication methods have not satisfied the requirements for tissue regeneration. The development of additive fabrication technologies, known as solid freeform fabrication (SFF), has made it possible to fabricate scaffolds with very fine structures and complex geometries using computer-aided design (CAD) data acquired from medical images of patients. Due to the advantages of SFF technology, it is rapidly becoming the technique of choice for scaffold fabrication. Moreover, recent research has demonstrated that a variety of biomaterials are suitable for use in various SFF systems. This paper reviews the application, advancement, and potential of SFF technologies in the fabrication of scaffolds for tissue regeneration.

Introduction

To restore and regenerate defective tissues and organs, transplantation (e.g., autograft, allograft, and xenograft) and biomaterial implantation (e.g., prosthesis) are promising therapies. However, each has limitations. Organ transplantation requires additional surgery to obtain a graft, risking infection caused by the host graft site and potentially graft supply shortages. Biomaterial implants often integrate poorly with host tissue and fail due to fatigue/wear in the human body.1,2

To overcome such limitations, tissue engineering was introduced. First defined by Skalak and Fox (1988)2–4 as “the application of principles and methods of engineering and life sciences toward the fundamental understanding of structure–function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve tissue function,” tissue engineering is a multi-disciplinary field that aims to develop...
artificial tissues for the regeneration of defective tissues. To regenerate three-dimensional (3D) organs or tissues using tissue engineering, a porous and biodegradable 3D structure known as a scaffold, including biofactors (cells and/or growth factors), is transplanted into the defective site. The scaffold, made of biomaterial, delivers the biofactors, maintains the cell volume, and provides structural support during tissue regeneration. To fabricate ideal scaffolds for tissue regeneration, several requirements must be satisfied (Table 1).

Conventional scaffold fabrication methods include solvent casting, particulate leaching, gas foaming, fiber meshes/fiber bonding, phase separation, melt molding, emulsion freeze drying, solution casting, and freeze drying. Although the fabrication processes are relatively simple, these methods do not control the size, shape, distribution, or interconnectedness of pores. In addition, organic solvents such as chloroform or methylene chloride are used to dissolve the synthetic polymers. After scaffold fabrication, the presence of organic solvent residues can be toxic to cells. Thus, many researchers agree that there is a need for advanced scaffold fabrication methods to overcome the limitations of conventional methods. Solid freeform fabrication (SFF) is a viable alternative.

**SFF technology**

SFF is a manufacturing technique that produces complex 3D structures by selectively adding materials. With SFF, two-dimensional (2D) patterns that represent the cross-section of the 3D structure are stacked layer by layer. This method controls the scaffold design parameters, such as the size, shape, distribution, and interconnectedness of pores. Moreover, it can be used to fabricate customized scaffolds from medical image data such as magnetic resonance imaging (MRI) and computerized tomography (CT) because it is based on computer-aided design (CAD). In addition, it can be used to fabricate scaffolds with high reproducibility. SFF is categorized into stereolithography (SL), fused deposition modeling (FDM), and selective laser sintering (SLS) (Fig. 1).

SL produces 3D scaffolds via selective photocuring of a photopolymer. Ultraviolet (UV) laser irradiation on the surface of the photopolymer causes solidification. UV laser scans generate 2D patterns, and the desired 3D structure is formed by stacking the solidified 2D patterns together. To improve resolution, micro-stereolithography (MSTL) and nanostereolithography (NSTL) were developed from SL using a specific laser system to fabricate 3D structures on micrometre and nanometre scales. Although this technique has the highest resolution among SFF technologies, it requires photocurable materials, which are limited in supply and costly.

FDM extrudes materials (generally thermoplastic) through a nozzle directly onto the build platform and fabricates 3D scaffolds by stacking 2D patterns. A general FDM system utilizes a filament from liquefied material by two rollers. However, this step needs additional time for fabrication. To remove this unnecessary time consumption, novel FDM, which is called as precision extrusion deposition (PED), was developed to use bulk materials in granulated form. This technique does not require solvents and offers ease and flexibility in the handling and processing of materials compared to other SFF technologies.

SLS uses a CO2 laser to selectively heat and sinter various materials, including polymers, ceramics, metallic powders, and their composites just below their melting points. The laser scans the shape of a cross-section of the 3D structure onto the surface of a powder bed. Then a new layer of powder is deposited by a roller, and the process is repeated. SLS can create complex structures, such as anatomically shaped scaffolds, with controlled pore size, porosity, and topology more conveniently than other SFF methods.

**Application to tissue engineering**

SFF has several advantages for 3D scaffold fabrication due to its advantages as a powerful manufacturing tool. The outer shape of the scaffold can be precisely reconstructed based on 3D CAD data of the target tissue or organ, which are converted from medical CT or MRI images. The layer-by-layer SFF enables control of the internal architecture of the scaffold (Fig. 2).

Initially, the types of biomaterials that could be used directly with SFF were limited. Many researchers have attempted to address this problem by studying the fabrication conditions of Food and Drug

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**Table 1 Requirements for fabrication of tissue scaffolds**

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<th>Requirement</th>
<th>Description</th>
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<tr>
<td>Biocompatibility</td>
<td>Non-toxic materials suitable for cell attachment, proliferation, and differentiation</td>
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<tr>
<td>Biodegradability</td>
<td>Degradable materials and adjustable degradation rate to match the rate of tissue regeneration</td>
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<tr>
<td>Porosity</td>
<td>Porous structure and appropriate interconnectivity for tissue integration, vascularization, and transportation of nutrients and oxygen</td>
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<tr>
<td>Structural support</td>
<td>Sufficient strength to withstand stress in the host tissue environment</td>
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Administration-approved biomaterials and developing various fabrication techniques for SFF systems as well as synthesizing new biomaterials. For photopolymerization via UV laser light in SL, MSTL, and NSTL, photocurable biomaterials such as polypropylene fumarate (PPF)\(^\text{33,37,38}\) and materials based on trimethylene carbonate (TMC)\(^\text{32,39}\) have been developed. Scaffolds fabricated using PPF and MSTL are precise (to a few tenths of a micron), and the cell adhesion and proliferation capabilities can be significantly enhanced through surface modifications such as biomimetic apatite coating and the attachment of various peptides.\(^\text{33}\) Scaffolds fabricated with PPF and a bone morphogenetic protein 2 (BMP-2) mixture have shown promise for bone tissue regeneration\(^\text{18}\) (Fig. 3). In these experiments, traditional scaffolds, BMP-2-unloaded SFF scaffolds, and BMP-2-loaded SFF scaffolds were implanted into an 8 mm diameter bony defect in the central part of the rat cranial bone (Fig. 3c, f and i). The results showed that new bone formation in the BMP-2 loaded SFF scaffolds was markedly better than that in the BMP-2 unloaded scaffolds (Fig. 3j and k). Moreover, the SFF scaffolds fabricated using MSTL were superior to traditional scaffolds fabricated using the particulate leaching/gas foaming method (Fig. 3d–h). NSTL has been used to fabricate bi-pore scaffolds with extremely precise pores (a few microns in line width and a few tenths of a micron in line pitch) as well as global pores (a few hundreds of microns in line width and pitch), and with better cell adhesion and proliferation than scaffolds with only global pores.\(^\text{34}\) Combining SL with sintering enables the fabrication of scaffolds composed of ceramic materials with very complicated shapes.\(^\text{40}\) A mixture of photocurable polymer and bioceramic materials such as hydroxyapatite (HA) or tri-calcium phosphate (TCP) is used for direct fabrication of scaffolds in MSTL. Then the photopolymer is selectively removed during sintering at very high temperatures, while ceramic powders are bonded to each other. The development of a soluble photopolymer has exploited the potential of SL for most biomaterials.\(^\text{41}\) A 3D mold can be fabricated using MSTL with a soluble photopolymer followed by biomaterial injection. After dissolving the mold in

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**Fig. 1** Schematic diagram of SFF technologies: (a) stereolithography, (b) fused deposition modeling, (c) mini-extruder in precision extrusion deposition, (d) selective laser sintering (reproduced with permission from ref. 19, 24 and 25).

**Fig. 2** Scaffold fabrication by SFF technology: (a) flow chart of the process used to fabricate 3D scaffolds using CAD/CAM technology, (b) fabricated scaffolds with various biomaterials (reproduced with permission from ref. 32–36).
NaOH solution, the final scaffold is obtained with the reverse form of the mold. This molding process has been studied for use with various biomaterials such as poly-$\varepsilon$-caprolactone (PCL), bone cement, poly(lactic-co-glycolic) acid (PLGA), and chitosan-alginates for tissue engineering.\textsuperscript{35,42,43}

In FDM, 3D shapes are fabricated by extrusion of filament materials from a head-heated liquefier. FDM for preformed fibers with uniform size and material properties has rarely been reported for biomaterials. However, recent studies have reported scaffolds with a resolution of several tenths of a micron fabricated with various biomaterials such as polymer and blended polymer/bioceramic.\textsuperscript{24,36} In addition, an HA/PLGA conjugate scaffold with an intact BMP-2/PEG complex has been successfully fabricated for bone tissue regeneration.\textsuperscript{44}

The PED system has been also used to fabricate scaffolds with various biomaterials. Especially, for bone tissue regeneration, blended PCL/HA scaffolds have been fabricated and shown the higher compressive modulus and osteogenic gene expressions compared to PCL scaffolds.\textsuperscript{25}

SLS is the preferred fabrication process for producing ceramic structures but is limited to thermally stable polymers. Biocomposite blends of PCL with different percentages of HA have been sintered to assess their suitability for fabrication via SLS.\textsuperscript{35} SLS has been combined with conventional salt leaching to fabricate porous PCL scaffolds with a network of 3D branches and joining flow-channels.\textsuperscript{46}

Potential

The limited options of materials have been the major obstacle to SFF applications in tissue engineering. However, recent research has developed a wide variety of biomaterials that can be used in various SFF systems. This is the initial step to transfer SFF technology to tissue engineering, but future work should focus on taking advantage of SFF in terms of fabricating arbitrary 3D shapes with high resolution for achieving the ultimate goal of tissue engineering. The regeneration of relatively thin tissues such as skin has been successful, but challenges still remain for the regeneration of thick tissues and complex organs because of the difficulty of meeting metabolic needs and mimicking complex tissue. The mass-transport properties of porous scaffolds have been characterized, leading to the design of scaffolds with inner architectures for efficient oxygen/nutrient supply and waste removal.\textsuperscript{47–49}

Recent advances in SFF have enabled the fabrication of scaffolds designed with various biomaterials. Furthermore, dispensing-based SFF technology enables the direct writing of cells. Initially, commercial ink-jet printing heads were used to print cells in hydrogels without controlling their exact 3D positioning or mechanical support. However, multiple-nozzle SFF systems enable the fabrication of biomaterials/cell/growth factor hybrid structures, which are considered the ultimate pre-packaged tissue replacements, with exact 3D positioning (Fig. 4).\textsuperscript{2,50–58} In the near future, these attempts will enable us to regenerate not only bone but also complex tissues and organs, such as kidney and liver, using tissue engineering for clinical use. Although further research is necessary to improve the feasibility of SFF in hostile processing environments, these
technologies have shown potential for fabricating artificially regenerated organs.

Conclusions

SFF technologies, which are computer-based fabrication technologies using CAD/CAM, have shown potential for tissue engineering. SFF can fabricate 3D scaffolds from various biomaterials with controlled inner/outer architectures (pore size, pore geometry, orientation, interconnectivity, and anatomical shape) that are appropriate for the target tissue or organ. Using scaffolds fabricated by SFF, many researchers have shown improvements in tissue regeneration compared to results using scaffolds fabricated by conventional methods. Although SFF technologies have overcome the limitations of conventional methods and have several advantages for scaffold fabrication with various biomaterials, many challenges remain for regenerating complex tissues or organs with large volumes. Future developments in SFF will play a key role in achieving these goals.

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References


