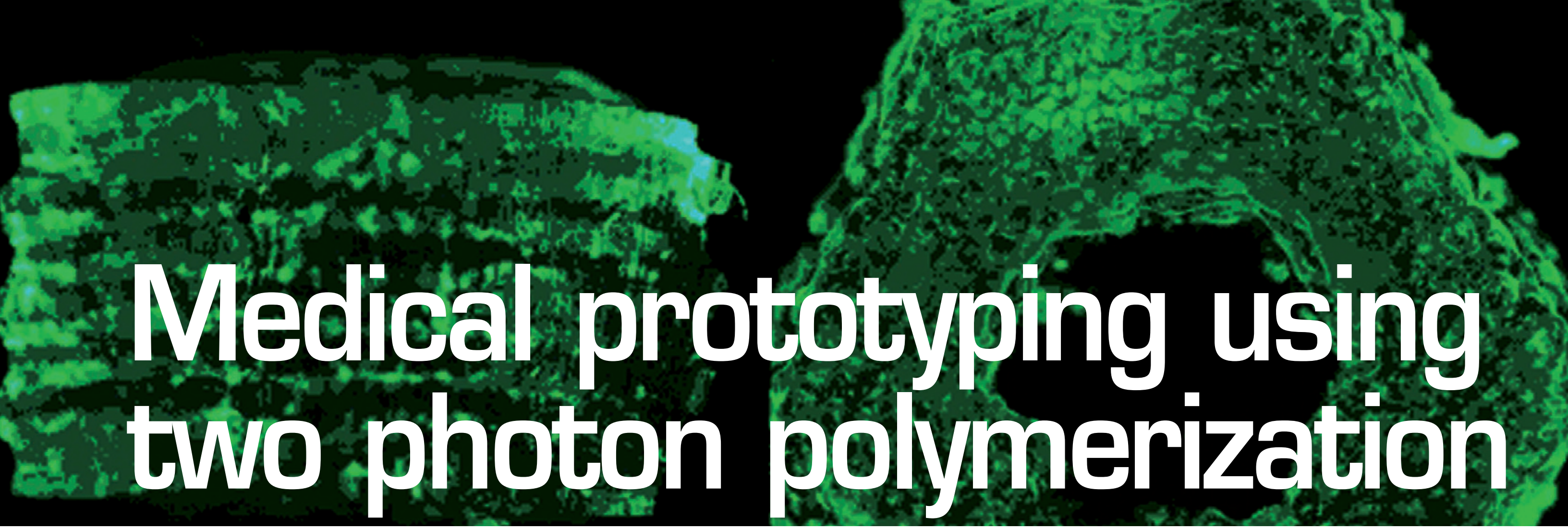

Medical prototyping using two photon polymerization

Two photon polymerization involves nearly simultaneous absorption of ultrashort laser pulses for selective curing of photosensitive material. This process has recently been used to create small-scale medical devices out of several classes of photosensitive materials, such as acrylate-based polymers, organically-modified ceramic materials, zirconium sol-gels, and titanium-containing hybrid materials. In this review, the use of two photon polymerization for fabrication of several types of small-scale medical devices, including microneedles, artificial tissues, microfluidic devices, pumps, sensors, and valves, from computer models is described. Necessary steps in the development of two photon polymerization as a commercially viable medical device manufacturing method are also considered.

Roger J Narayan[a*], Anand Doraiswamy[b], Douglas B Chrisey[c], Boris N Chichkov[d]

[a] *Joint Department of Biomedical Engineering, University of North Carolina and North Carolina State University, Raleigh, NC, USA*

[b] *Advanced Vision Science, Inc., Goleta, CA 93117, USA*

[c] *Department of Material Science and Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA*

[d] *Department of Nanotechnology, Laser Zentrum Hannover eV, Hannover, Germany*

* *E-mail: roger_narayan@unc.edu*

Rapid prototyping, also known as computer-aided manufacturing, layer manufacturing, and solid freeform fabrication, pertains to a set of materials processing technologies that dates to the late 1980s[1]. These technologies involve fabrication of a structure in the minimum possible time and with relatively few steps based on a three-dimensional computer-aided design (CAD) model. Rapid prototyping technologies commonly involve adding liquid, powder, or sheet precursor material in a layer-by-layer manner in order to process the desired structure along the Z-direction from bottom to top. Several steps are involved in the rapid prototyping of a structure, which include geometric modeling of the structure, converting the CAD model into a series of polygons that represent the surface of the structure, slicing the facetted model into a stack of layers, and using the layer data for fabrication of the structure. There is a trade-off in rapid prototyping technologies between minimum feature size and processing time; use of thinner layers enables fabrication of more intricate shapes but requires longer processing times. According to Waterman *et al.*, rapid prototyping technologies may reduce time to market by 90% and costs by up to 70%[2]. Indirect approaches may also be used, in which rapid

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prototyping is used to create a pattern, which is subsequently used for molding or investment casting of the desired structure[3].

Rapid prototyping technologies are of great interest for medical prosthesis and medical device applications. Magnetic resonance imaging, micro-computed tomography, or other medical imaging modalities may be used in conjunction with rapid prototyping in order to create patient-specific implants or evaluate novel prosthesis designs[4]. For example, Webb *et al.* described the use of computed tomography data on a bone defect to create a patient-specific craniomaxillofacial implant[4]. In addition, rapid prototyping methods enable processing of structures with complex internal geometries (e.g., catheters)[3]. Rapid prototyping technologies may also be used to prepare porous biodegradable materials for use as scaffolds in artificial tissues[5]. Cooke *et al.* utilized stereolithography to create structures out of a biodegradable resin containing poly(propylene fumarate), diethyl fumarate (DEF), and bisacylphosphine oxide[6]. Sun *et al.* noted that topology, surface chemistry, pore connectivity, and pore size affect scaffold performance parameters such as nutrient transport, cell attachment, and cell ingrowth[7]. For example, small-scale structures on the scaffold surface may serve to increase surface area, increasing adsorption of proteins and attachment of cells[8]. Scaffold performance is also related to elastic modulus, strength, and other mechanical properties[7]. Several investigators, including Sun *et al.* and Smith *et al.*, have noted that scaffold fabrication methods should allow for (a) the processing of structures with overall dimensions larger than 1 cm; (b) the processing of structures with features smaller than 1 μm; (c) the processing of several biocompatible materials in a single procedure; and (d) the incorporation of proteins (e.g., growth factors), oligosaccharides, nucleic acids, and cells during processing[7,9].

Stereolithography is a liquid-based rapid prototyping technology that was invented by Charles Hull of 3D Systems Inc. approximately twenty-five years ago[10]. In this technology, a liquid resin containing low molecular weight monomers is cured in a layer-by-layer manner in order to form a solid polymer structure. By moving the focus of the argon ion laser or helium cadmium laser in X- and Y- directions, single photon absorption enables polymerization of a photosensitive liquid (e.g., an acrylic or epoxy resin) in two dimensions. The liquid is located on a platform, which is lowered after solidification of each layer. The completed structure is subsequently exposed to ultraviolet light in a fluorescent oven for complete polymerization of the solid. The minimum feature size is determined by the laser spot size; the minimum layer thickness commonly achieved using this technology is 50 μm[3]. Melchels *et al.* recently utilized a stereolithography apparatus containing a digital micro-mirror device in order to fabricate porous poly (d,l-lactide) network scaffolds[11]. The minimum feature size was determined by the overcure depth (7 μm) as well as instrument-specific parameters, such as layer thickness (25 μm) and pixel size (32 × 32 μm^2). The open (gyroid) architecture of the scaffolds facilitated penetration of water as well as the attachment of murine MC3T3 pre-osteoblast cells in a homogeneous distribution. Rapid Micro Product Development (RMPD®) is another laser-based process for the rapid prototyping of photosensitive materials, including acrylic and epoxy resins[12]. Structures with layer thicknesses of one micrometer and overall dimensions of 50 mm x 50 mm x 50 mm may be prepared using this approach.

How two photon polymerization is performed

Two photon polymerization (2PP) is a liquid-based rapid prototyping technology that has been utilized for the fabrication of small-scale three-dimensional medical devices from computer designs in recent years[8]. In 1931, Maria Goeppert-Mayer theoretically described the phenomenon of multi-photon absorption, a process in which interactions between many photons and an atom or a molecule take place during a single quantum event[13]. Kaiser and Garrett experimentally demonstrated two photon excitation in 1961. In their work fluorescent blue light (with a wavelength of 425 nm) was obtained by illuminating $CaF_2:Eu^{2+}$ crystals with light from a ruby laser (with a wavelength of 694 nm)[14].

In two photon polymerization, nearly simultaneous absorption of two photons within a small volume in a photosensitive resin induces chemical reactions between photoinitiator molecules and monomers. A laser capable of generating femtosecond pulses is commonly used to create high energy intensity in the focal volume. In particular, femtosecond titanium:sapphire lasers operating at approximately 800 nm wavelength are used for 2PP due to their short pulse width and high peak power[15]. Absorption of 800 nm photons in a nearly simultaneous manner by the photoinitiator is a quantum event whose energy corresponds to the ultraviolet light region of the electromagnetic spectrum[16,17]. Photoinitiator resins with sensitivity to 390 nm wavelength radiation are commonly utilized. Most materials that are commonly used in 2PP were originally utilized as negative photoresists in conventional ultraviolet photolithography, including an epoxy oligomer-containing commercial photoresist (SU-8) and organically-modified ceramic material[18].

A voxel (volumetric pixel) is defined as the unit volume of material cured by 2PP. The minimum feature size is dependent on several processing parameters, including exposure time, laser power, numerical aperture of the objective lens, sensitivity of the resin, and voxel-voxel distance[19,20]. In order to perform layer-by layer processing of three-dimensional structures, the voxel of polymerized material must exhibit a low aspect ratio. Voxels with low aspect ratios may be achieved through the use of low laser power and low exposure time at an energy that is near the threshold energy. The laser beam is focused into the volume of a near infrared light-transparent photosensitive resin[19,20]. The scanning path of the laser focus is commonly moved along a two-dimensional scanning path, while translation of the beam spot in the Z-axis enables processing of three-dimensional structures.

Devices may be created by means of either contour scanning or raster scanning. In the contour scanning approach, the laser beam solidifies the contour of the structure; the bulk of the structure is solidified in a post-two photon polymerization step. The raster scanning approach involves scanning the laser beam over the entire volume of the structure. Contour scanning enables structures to be fabricated in a more rapid manner. It should be noted that deformation of thin-walled structures created by means of the contour scanning method may occur due to surface tension and flow variations during developing; this issue may be overcome by increasing the thickness of the polymerized structure. The washing away of unpolymerized material is the only step that is required after either two photon polymerization approach.

Solidification of material in 2PP occurs over a highly localized volume due to the quadratic dependence of the two photon absorption probability on intensity[16]. Maruo *et al.* first demonstrated processing of three-dimensional microscale structures, including 7 μm diameter spiral structures, out of a urethane acrylate resin[21]. Features with lateral resolutions below 100 nm have previously been demonstrated using 2PP. For example, Takada *et al.* demonstrated fabrication of structures with a lateral spatial resolution of approximately 100 nm by incorporating radical quenchers within the photosensitive resin[22].

Two photon polymerization may be used to process a variety of photosensitive materials, including acrylate-based polymers, organically-modified ceramic materials, zirconium sol-gels, and titanium-containing hybrid materials; many of these materials are widely available and may be obtained at low cost[23-25]. In addition, 2PP equipment can be placed in a conventional environment; no clean room facilities or other specialized facilities are required. Finally, the processing rates of 2PP and particularly the indirect processes based on 2PP are suitable for translation to high-rate commercial manufacturing. A variety of soft lithography approaches may be used for high fidelity replication of microscale and nanoscale structures created by means of 2PP. For example, microcontact molding, microcontact printing, microtransfer molding, membrane assisted microtransfer molding, and replica molding may be used to replicate two photon polymerization-fabricated structures[26,27].

Medical applications of two photon polymerization

Due to these unique capabilities, two photon polymerization has been used to create a variety of medical devices with small-scale features. For example, the technique has been used to fabricate microneedles, which are lancet- or thorn-shaped devices for transdermal delivery of pharmacologic agents or sampling of body fluids (e.g., blood). Microneedles with complex shapes may also be fabricated[28], such as those with a rocket-like geometry (Fig. 1). This geometry, which consists of a cylindrical shaft and support braces, exhibits a relatively small tip angle. Smaller microneedle tip angles are associated with lower microneedle penetration forces[29]. Gittard *et al.* used a combination of 2PP and polydimethyl siloxane micromolding to fabricate microneedle arrays out of a photoreactive acrylate-based polymer[30]. A master structure for the 5 x 5 solid microneedle array (with a height of 500 μm and base diameter of 150 μm) was created using 2PP. This structure was subsequently used to make a negative mold from poly(dimethyl siloxane); photoreactive acrylate-based polymer microneedle arrays were cast using this negative mold. The microneedle arrays withstood a ten Newton axial load without fracture, and were also shown to successfully penetrate human stratum corneum and epidermal tissue that was obtained from a surgical abdominoplasty. Their work suggested that two photon polymerization-micromolding is a scalable technique, which may be used to create solid microneedles and other solid devices out of a broad range of materials (e.g., non-infrared transparent materials).

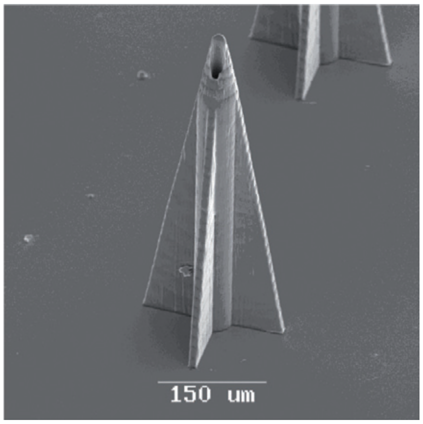

Fig. 1 Scanning electron microscopy image of a rocket-shape microneedle, which was fabricated using two photon polymerization. The small cross-sectional area of this design minimizes the skin penetration force. Reproduced from[2] with permission of Informa Healthcare. © 2010 Informa Medical & Pharmaceutical Science.

Gittard *et al.* recently fabricated hollow microneedles with heights between 500 and 700 μm out of an acrylate-based polymer, which is used in Class IIa medical devices such as hearing aid shells (Fig. 2)[31]. These devices were used to create pores in the stratum corneum layer of cadaveric porcine skin, which enabled carboxyl quantum dots to be distributed in the deep epidermis and dermis within fifteen minutes. Quantum dots are fluorescent semiconductor nanoparticles with diameters between 2 and 10 nm, which may be conjugated with peptides, antibodies, aptamers, pharmacologic agents, and other tumor-specific molecules in order to allow for imaging of neoplastic tissue. On the other hand, topically-applied carboxyl quantum dots demonstrated poor penetration and remained on the topmost 50 μm of the cadaveric porcine skin. These studies indicate that 2PP enables rapid iteration of microneedle design parameters (e.g., microneedle length), which may facilitate the use of microneedles in clinical applications.

Small-scale microscale devices with moving parts have also been created using 2PP. For example, Schizas *et al.* processed micro check valves with lengths of 360 μm, internal valve diameters of 70 μm, and external diameters of 120 μm out of a photosensitive zirconium-silicon sol-gel[24]. These valves contained internal moving parts; movement of the piston rod was demonstrated using a needle. It is interesting to note that these structures did not exhibit stair-step surface features despite the fact that they were processed in a layer-by-layer manner. Devices with similar diameter values could be used to replace natural

(a) (b) (c)

Fig. 2 Scanning electron microscopy images of acrylate-based polymer hollow microneedles, which were produced using two photon polymerization. (a) Image of microneedle array obtained at forty-five degree tilt. (b) Image of individual microneedle obtained at forty-five degree tilt. (c) Image of individual microneedle obtained at zero degree tilt. The lengths and base diameters of these microneedles are 508 +/- 33 μm and 212 +/- 3 μm, respectively. Dimensions are shown as average +/- standard deviation. Reproduced from[3], with permission from the Royal Society of Chemistry. © 2010 Royal Society of Chemistry.

check valves, which close under reverse fluid flow and open under forward fluid flow in order to minimize reverse blood flow in human veins[18]. Wu *et al*. created a microscale valve in a single step out of SU-8 resin by means of 2PP. This valve demonstrated movement from "off" to "on" positions based on water flow[32]. Such microscale valves may find use in "lab-on-a-chip" devices.

A microscale pump structure containing a channel, a film, and a solvent-responsive valve was created by Xiong *et al*. using 2PP[33]. In this study, a channel with an 8 μm tall, 12 μm wide, and 20 μm long entrance was created using polyacrylamide hydrogel. Bending of the hydrogel film and actuation of the pump was performed by alternating solutions between water (closed valve position) and ethanol (open valve position); a pump response time of 0.17 seconds was obtained. Reversibility of the pump was demonstrated by means of five solution exchanges between water and ethanol. Additional pump actuation approaches, including pH- and temperature-driven pumping, may be achieved using hydrogel materials.

It is also possible to produce light-responsive microscale structures using 2PP. Maruo *et al*. created constrained-type, optically-driven micromachines, such as micromanipulators and microturbines[17,34]. These devices are transparent to visible and infrared light and are capable of rotation, sliding, and swinging motions under focused laser irradiation due to a process known as optical trapping. They fabricated several devices, including a microturbine with a 14 μm diameter and a three-dimensional wheel; a multi-stage microscale gear system containing gears with 6.3 and 8.3 μm diameters; and a microscale manipulator with a 9.7 μm long, 1.7 μm wide arm. Rotation of the microturbine was achieved by circular scanning of a titanium:sapphire laser, which was operated in continuous wave mode over the device; a rotation rate of 4.6 rotations per minute was demonstrated. Motion of the micromanipulator under laser irradiation was also demonstrated. Rodrigo *et al*. created structures with interlocking functionality, including 13 μm long dumbbell structures and 17.5 μm long blocks with complementary features[35]. Components were assembled into structures by means of counterpropagating beam traps, which were created using a continuous wave fiber laser. These active microscale structures could find use in medical diagnostics applications, including the sorting of cells[34].

Microfluidic devices for "lab-on-a-chip" applications have been created; for example, Kumi *et al*. demonstrated high-speed two photon polymerization of microfluidic devices over centimeter-scale distances[36]. They used a fluorine-based photoacid generator in conjunction with SU-8 in order to create master structures for microfluidic devices with high aspect ratio features and uniform, rectangular cross-sections. Polydimethylsiloxane (silicone) molds with 20 μm wide channels and aspect ratios between five and ten were created from the SU-8 master structures. The 2PP method is particularly useful for creating microfluidic devices that cannot be readily fabricated by means of conventional photolithographic methods, including channels with non-rectangular cross-sections and structures with unusual flow profiles. Jariwala *et al*. demonstrated the fabrication of self-enclosed fluidic channels out of an organically-modified ceramic material[37]. Parallel pairs of ribs with heights of approximately 10 μm, wall thicknesses of approximately 3 μm, and channel widths of approximately 2 μm were created. The overlapping of subactivated regions of material caused polymerization of inter-rib material and formation of a closed channel. Rib width may be altered through modification of several parameters, including laser fluence and exposure time[38]. A 110 nm wide fluid channel was recently created by polymerizing multiple ribs of SU-8 in a parallel orientation[39]. Two photon polymerization is considered to be more rapid and less expensive than conventional lithography-based approaches for creating microfluidic devices.

Two photon polymerization has also been used to fabricate sensors for biological and medical applications out of composite materials. Drakakis *et al*. created three-dimensional grid structures out of organically-modified ceramic materials. Biotin was immobilized on these structures by means of ultraviolet-activated cross-linking. Binding of streptavidin was demonstrated using fluorescence microscopy and shear horizontal-surface acoustic wave monitoring[40]. Kim *et al*. created a glucose-sensing electrode for an electrochemical glucose sensor using 2PP[41]. In this case, the electrode material was a photosensitive material containing glucose oxidase, ferrocene, single-walled carbon nanotubes, an acrylate, an epoxy monomer, a photoinitiator, and a surfactant. The electrochemical detection of glucose was demonstrated using the nanotube-containing electrode; the nanotubes were shown to facilitate ferrocene activity in the electrode. These results suggest that 2PP enables rapid iteration of biosensor designs; in addition, composites containing photosensitive materials and biologically functional materials may readily be prepared.

In addition, two photon polymerization may be used to process cell-seeded scaffolds, which may be used to replace damaged or diseased tissues. In particular, the technique has been used to create scaffolds that guide cell development and provide mechanical support[42]. In 2004, Basu *et al*. described the cross-linking of composite protein structures containing fibrinogen, fibronectin, and concanavalin A; in this study, rose bengal was used as a photoactivator. Biological activity of cross-linked fibronectin and fibrinogen was demonstrated by means of immunofluorescence imaging[43]. Basu *et al*. subsequently described the use of two photon polymerization to create scaffolds for tissue engineering using collagen types I, II, and IV as well as a benzophenone dimer photoactivator[44]. 780 nm and 850 nm wide structures were fabricated out of collagen types I and II using three photon and two photon absorption, respectively. Bioactivity of the cross-linked collagen structures was established using immunofluorescence. In addition, human dermal fibroblast cell attachment to linear structures was demonstrated by means of phase contrast imaging. It should be noted that Jhaveri *et al*. associated this protein cross-linking technique with several shortcomings, including long exposure times and use of high energies[45].

A Lego®-like structure with interlocking pillar features has been fabricated using organically-modified ceramic material by Doraiswamy *et al*.[46]. The scaffold contained arrays of cylindrical pillars (with a diameter of 75 µm and a height of 20 µm) on both sides of a flat chip. B35 neuroblast-like cells were seeded on these structures in *in vitro* studies; these cells were shown to orient along the pillar walls and increase in number over time. 100% of the B35 neuroblast-like cells remained viable forty-eight hours after seeding. Layer-by-layer stacking of scaffolds into three-dimensional tissues may be enabled by pillar interlocking. Ovsianikov *et al*. created scaffolds using Ormocomp® organically-modified ceramic material[47]. Vertical surfaces of organically-modified ceramic material were shown to support growth of granulosa, endothelial, neuroblastoma, and Chinese hamster ovary cells. Tayalia *et al*. utilized two photon polymerization to create interconnected woodpile scaffolds out of a viscous triacrylate two-monomer material[48]. By altering the relative amounts of the two monomers in the material, the elastic modulus was varied between 0.1 and 1.2 GPa. Fibrosarcoma cell migration over two-dimensional and three-dimensional scaffolds was observed. They noted that cell migration over three-dimensional scaffolds was more rapid than over two-dimensional scaffolds. In addition, a reduction in scaffold pore size was associated with a decrease in the cell migration rate.

Three-dimensional structures were created using 2PP out of a biodegradable polycaprolactone-based triblock copolymer by Claeyssens *et al*.; 4,4'-bis(diethylamino)benzophenone was utilized as a photoinitiator in this study[49]. Live-dead cell staining studies with 3T3 fibroblast cells indicated that these materials did not affect cell viability. Hidai *et al*. prepared 330 µm long, 6-9 µm diameter fiber scaffolds out of organically-modified ceramic material using 2PP[50]. Structures grew from the laser focus to the light source by means of accumulation, self-focusing, and self-growing; no scanning of sample or the lens was performed. Elongation and alignment of epithelial cells and fibroblast cells on fibronectin-coated fibers with various orientations was demonstrated.

Scaffolds have also been created out of poly(ethylene glycol) hydrogels. For example, a three-dimensional scaffold fabricated by Ovsianikov *et al*. out of poly(ethylene glycol) diacrylate material with a molecular weight of 742 is shown[51] in Fig. 3. In this structure, cylinders with heights of 50 µm and radii of 25 µm are stacked such that the cylinders in a given layer partially overlap the cylinders in the underlying layer. Structures with features as small as 200 nm were prepared using poly(ethylene glycol) diacrylate materials with molecular weights of 302 and 742. Jhaveri *et al*. fabricated structures out of 2-hydroxyethyl methacrylate (HEMA) and poly(ethylene glycol) diacrylate (PEG-DA) synthetic hydrogels using an FDA-approved chromophore and a conventional photoinitiator (Irgacure 651) in aqueous solutio[45]. The hydrogel structures remained attached to silanized glass substrates after multiple swelling-deswelling cycles. These results indicate that hydrophobic chromophores facilitate two photon polymerization of hydrogel scaffolds by means of intermolecular electron transfer and/or photosensitization processes.

Biologically-relevant materials and cells have recently been incorporated within two photon polymerization-fabricated scaffolds. For example, Ovsianikov *et al*. utilized a combination of 2PP and laser-induced forward transfer to fabricate poly(ethylene glycol) diacrylate scaffolds and seed the scaffolds with cells, respectively (Fig. 4)[51,52]. Endothelial cells were seeded on the inner region of the scaffold and vascular smooth muscle-like cells were seeded on the outer region of the scaffold by means of laser-induced forward transfer; a sharp boundary between the smooth muscle cell-seeded region and endothelial cell-seeded region was observed using fluorescent microscopy. Hoffman *et al*. showed two photon absorption-based patterning of three-dimensional structures containing fibronectin-derived peptides (RGDS and CS-1) within a polyethylene glycol diacrylate hydrogel matrix[53]. A relationship between RGDS concentration and laser processing parameters was

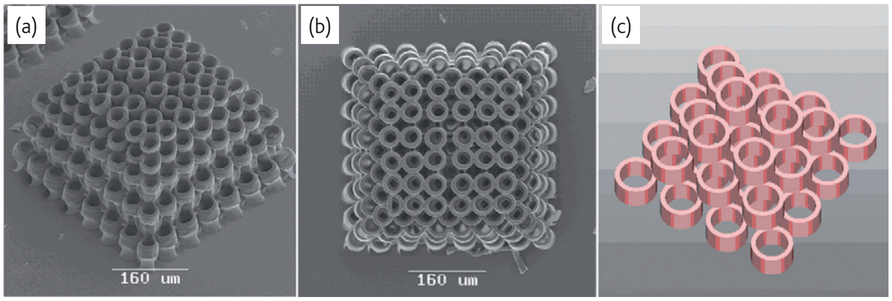

Fig. 3 Three-dimensional scaffold fabricated by two-photon polymerization of SR610 material: (a) perspective view SEM image of produced structure; (b) top view SEM image of produced structure; (c) schematic illustration of cylinder arrangement. Figure reproduced from[51] with permission of Elsevier.

demonstrated; cross-linking of RGDS molecules was directly correlated with laser intensity and laser scan sped. These efforts suggest that scaffolds fabricated using 2PP may be used to form the basis of multilayered, heterogeneous artificial tissues.

In recent work, Hsieh *et al*. demonstrated two photon processing using a scan speed of 30 mm/s[54]. They created a 2.5 mm x 2.5 mm x 2.5 mm cubic scaffold out of a commercially-obtained photocurable polymer in 2 hours; seeding of HepG2 (liver cancer cell line) cells as well as primary hepatocytes on the scaffold was demonstrated. In addition, primary hepatocytes cultured within the cubic scaffold demonstrated higher liver-specific activity than primary hepatocytes cultured on a two-dimensional surface. The relatively rapid processing rate described in this study may facilitate the use of 2PP for creating scaffolds with clinically-relevant dimensions.

Two photon polymerization may be used to create chemically and/or physically heterogeneous surfaces. For example, Pins *et al.* used multiphoton excitation to create 200 µm long, 600 nm wide linear structures containing several proteins, including fibronectin, fibrinogen, and bovine serum albumin. Cells adhered to bovine serum albumin structures experienced a constraint in cell spreading as compared with cells adhered to fibrinogen structures and fibronectin structures[55]. Jeon *et al*. fabricated a variety of patterns, including orthogonal mesh structures and parallel line structures, by means of 2PP[56]. Parallel lines with heights between 0.5 and 1.5 µm as well as orthogonal mesh structures with heights between 4 and 6 µm were prepared. Cell behavior was observed using phase contrast and fluorescent microscopy. Cells on the parallel ridge structures demonstrated high length:width ratio morphologies and deformed into elliptical shapes. The cell aspect ratio was shown to increase with ridge height; furthermore, the threshold height for cell alignment was shown to be approximately 1 µm.

More recently, Jeon *et al*. fabricated anisotropic cross patterns and parallel line patterns out of organically-modified ceramic material in order to examine migration and morphology of NIH/3T3 fibroblast cells[57]. Structures with heights of 3 and 10 µm, ridge widths of between 1 and 2 µm, and grid aspect ratios of 1:2 and 1:4 were examined. Microscale patterns were shown to influence cell parameters, including orientation, migration, and attachment. The effect of pattern geometry on cell alignment and motility decreased as the distance between the lines increased. In addition, cross patterns were shown to increase the cell migration rate. Cross patterns were more effective than parallel line patterns in modulating the cell migration rate. Furthermore, lower ridges were shown to have a greater influence on the cell migration rate than higher ridges. These studies demonstrate the utility of 2PP for creating surfaces with well-defined small-scale surface features, which may be used to modulate cell activities such as adhesion, differentiation, proliferation, and spreading[55].

Zhang *et al.* evaluated the use of 2PP for creating nanoimprinting molds out of dipentaerythritol pentacrylate and Irgacure 819 photoinitiator; structures containing parallel lines with widths of 400 nm, heights of 1.2 µm, and inter-line distances of 5 µm were created[58]. Patterns of poly(ethylene glycol) diacrylate, a material that may be used in artificial tissue scaffolds, were imprinted. Molding was accomplished by means of a mask aligner; silanization was used to enable detachment of the dipentaerythritol pentacrylate mold from the poly (ethylene glycol) pattern. A high degree of fidelity between the dimensions of the poly (ethylene glycol) diacrylate pattern and the dipentaerythritol pentacrylate

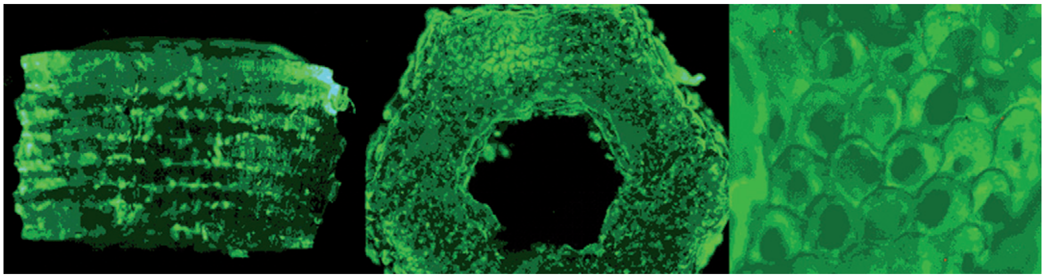

Fig. 4 Polyethylene glycol scaffold created using two photon polymerization and seeded with cells using laser induced forward transfer. Courtesy Prof. B. Chichkov (Laser Zentrum Hannover).

nanoimprinting mold was demonstrated over a large area. The authors suggested that molded poly(ethylene glycol) diacrylate structures may find use as scaffold materials in artificial tissues.

Two photon polymerization has been used to fabricate small bone prostheses with microscale features. For example, Ovsianikov *et al*. demonstrated fabrication of prostheses for the middle ear bones, known as ossicular replacement prostheses, out of organically-modified ceramic material[59]. This structure was similar to that of a total ossicular replacement prosthesis manufactured by Kurz Medical Inc. (Dusslingen, Germany). Conical structures were fabricated on the prosthesis head; these structures may serve to decrease the likelihood of tympanic membrane perforation and improve cell adhesion. These results suggest that 2PP may be used to create prostheses with microscale surface features.

Conclusions

Two photon polymerization has been used to prepare a variety of small-scale structures for medical applications. Several private companies, governmental organizations, and universities are currently involved with fabricating and evaluating 2PP-fabricated medical devices. A variety of considerations, including translation- and commercialization-related challenges, will need to be considered over the coming years. Increasing the 2PP processing rate by means of multibeam parallel processing and other mechanisms is being considered[18]. For example, parallel processing of structures through the use of a microlens array, which serves to transform a collimated beam into multiple focus spots, may be used to increase throughput[19]. This method may facilitate large-scale processing of identical structures. In addition, the development of novel organic π-conjugated chromophores may enable 2PP using continuous wave or nanosecond pulsed lasers[19]. A limited number of photosensitive polymers has been processed using 2PP; expanding the number of two photon polymerization-compatible materials, particularly biodegradable materials and materials containing biologically active molecules, would be of benefit[54]. Refinement of 2PP processing parameters for each specific application is also needed. Additional studies to examine the mechanical behavior of two photon polymerization-fabricated structures need to be performed. In addition, sterilization processes for two photon polymerization-fabricated devices, including those containing temperature-sensitive biological materials, must be developed[8]. Finally, 2PP needs to become cost competitive with conventional machining- and lithography-based methods. If these obstacles can be overcome, two photon polymerization may achieve commercial importance in medical device fabrication over the coming decades. mt

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