Chapter 18
3D Photopolymerization for Biofabrication

Nadine Nottrodt and Elke Bremus-Köbberling

The chemical process of photo-induced polymerization has found widespread applications in the field of 3D printing. A special field of applications is biofabrication where photopolymerization is used for 3D bioprinting, e.g., for tissue engineering. In brief, a photopolymerization reaction uses the energy of light to initiate the cross-linking of monomers or pre-polymers to form polymeric networks. The prevalent mechanism of chain growth is radical polymerization of acrylic monomers. This reaction is typically started via UV-irradiation (< 400 nm) and requires a photoinitiator (PI) to produce the reactive species. Nowadays other mechanisms like cationic or "living" polymerizations or thiol-ene click reactions come more and more into focus (Bagheri et al. 2019; Chatani et al. 2014). In academic as well as in industrial research and development 3D photopolymerization-based technologies like stereolithography (SL), digital light processing (DLP), and multiphoton polymerization (MPP) are widely applied to fabricate components and devices with special mechanical, optical, chemical, or biological properties. For biofabrication additional properties like cytotoxicity of monomers and photoinitiators as well as UV radiation being harmful for cells have to be taken into account. New materials and technologies for biofabrication purposes have to be elaborated.

18.1 Stereolithography (SL)

Stereolithography is one of the first rapid prototyping technologies using a photo resin to build three-dimensional structures by layer-wise UV light irradiation. In 1984, Chuck Hull invented the first stereolithographic machine (US patent US4575330A)

N. Nottrodt (✉) · E. Bremus-Köbberling
Fraunhofer Institute for Laser Technoloy ILT, Steinbachstrasse 15, 52074 Aachen, Germany
e-mail: nadine.nottrodt@ilt.fraunhofer.de

which was able to fabricate 3D printed components. A very promising field of application for SL is the evolving field of biofabrication. In 2009, Mironov et al. gave a definition on Biofabrication, which explains that Biofabrication means the production of complex living and non-living biological products from raw materials such as living cells, molecules, extracellular matrices, and biomaterials (Mironov et al. 2009). As a production tool for three-dimensional structures, SL is very promising for tissue engineering by using materials like cells and tissue-relevant photo-cross linkable materials to build scaffolds for cell embedding.

The general setup of a stereolithographic machine (Fig. 18.1) contains a vat, a building platform, and a light source. The vat is the reservoir for the photo resin and the building chamber at the same time. Photo resins consist of monomers with either photo-reactive side chains, e.g., moieties like acrylate groups in combination with a special photoinitiator (PI) sensitive for a chosen UV light or thiol-ene moieties which can be polymerized directly by UV light (266 nm) (Hoffmann, et al., 2017). Typical PIs react at wavelengths in the range of 350 to 400 nm, e.g., Irgacure 2959 or 369 (Stampfl et al. 2008; Gauvin et al. 2012). For biofabrication application, the cytocompatibility of PIs is highly important. The mentioned Irgacure materials have been positively tested for their cell compatibility, nevertheless UV light is harmful for the cell DNA. Therefore other PIs working in the near UV (400–490 nm) like lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) or even visible light sensitive PIs are also under investigation and show promising results for biofabrication.

Two different setups for SL can be distinguished; one is a bottom up, the other one a top-down approach. In a bottom-up approach, the UV light comes from the top of the vat, while the light in a top-down approach is coupled into the vat through a transparent window in the bottom of the vat. During a bottom-up printing process,
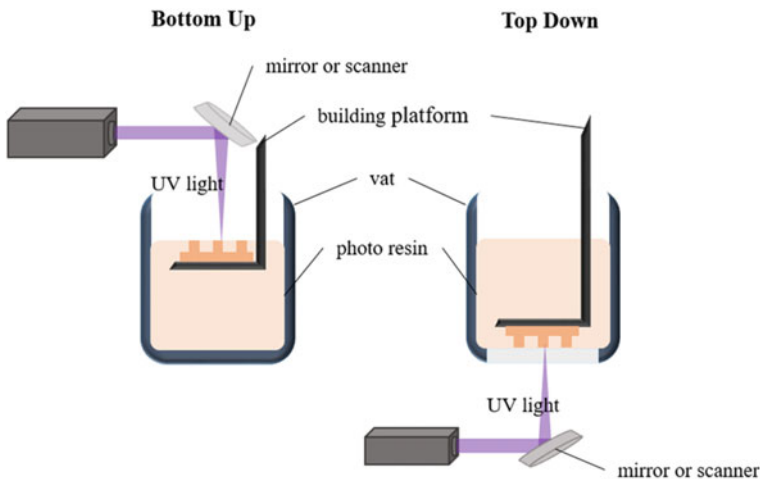

Fig. 18.1 The general stereolithographic setup consists of a vat, a building platform, and a light source. The main difference between the bottom-up and top-down approach for stereolithography is determined by the direction of irradiation

the building platform is positioned several micrometers below the surface of the resin. During the next step, the photo resin is exposed to the UV light and the material starts to polymerize. Afterward the platform lowers to allow the resin to cover the surface equally either by surface tension or a coating setup and irradiation continues. This procedure is repeated several hundreds of time to allow for a layer-by-layer 3D printing. Within the top-down approach, the platform is allocated at the bottom of the vat. The distance between the platform and the bottom defines the layer thickness. After irradiation the platform lifts up from the bottom, resin can fill the gap and another irradiation process can start to build the 3D object. The latter has the advantage that oxygen inhibition is prohibited.

While classical SL uses laser irradiation to induce single photon induced photo cross-linking with the limitations of being restricted to the surface due to low penetration depth into the liquid monomer mixture and consecutive polymerization steps with uncontrolled dark reactions, two further processes will be described. Digital light processing (DLP) to increase the building rate and multiphoton polymerization (MPP) for direct laser writing (DLW) to increase the printing resolution.

18.2   Digital Light Processing (DLP)

The main idea of DLP technology follows the top-down approach of SL taking the advantage of an illumination system at 405 nm wavelength using projection to illuminate the printing platform. The light is guided on a digital mirror device (DMD) which is a dynamic mask consisting of microscopically small mirrors on a semiconductor chip, which can be switched on and off rapidly and guide the light on small lenses focusing on the building platform. Doing so, a whole layer can be photocured in once and not point-by-point compared to SL. Therefore the building rate is enhanced. Since the projector uses a screen for projection, each voxel has cubic shape, which decreases the resolution of the printing product in x–y-direction. The resolution in z-direction depends on the layer thickness independent from SL or DLP process.

18.3   Multiphoton Polymerization (MPP)

Multiphoton lithography or multiphoton polymerization for direct laser writing (DLW) describes a method able to write structures in polymerizable resins with a resolution < 100 nm. The first idea of two photon absorption (TPA)—i.e., simultaneous absorption of two photons of a longer wavelength is effective to overcome the same energy barrier as one photon of twice the energy—was depicted by Maria Göppert-Mayer in the early 1930s (Herzfeld and Göppert-Mayer 1931). However, it took until solid-state femtosecond lasers became available (Moulton 1982) that a wider scientific community received the feasibility of two photon processes. Since

then this topic is being extensively investigated which can be seen from current reviews and papers (Ovsianikov et al. 2012; Whitby et al. 2017; Stokker-Cheregi et al. 2018).

The multiphoton polymerization process in DLW is based on two effects: first ultrashort laser pulses in the femtosecond (fs) range and second a tight focusing of the laser beam (NA > 1). These simultaneous effects allow for very high photon density in the focus causing non-linear effects (Miwa et al. 2001).

When focusing a UV laser onto a photo resin each single photon can start the polymerization reaction during SL immediately, resulting in polymerization beginning at the surface and continuing along the beam path into the photo resin limited by the optical penetration depth. The resolution also depends on the polymer layer thickness. In comparison (Fig. 18.2) in MPP, a tightly focused laser beam with ultrashort laser pulses at a wavelength in the visible region of the spectrum is focused on the volume of the transparent photo resin. Only the intensity in the focus is high enough to excite the resin by multiphoton absorption. The absorption causes a chemical change in a small volumetric pixel (voxel). The size of the voxel is determined by laser power and focal width and defines the resolution. By moving the laser focus a second voxel is produced next to the first one. An overlapping distance between the voxels results in a line. Diffusion effects in the irradiated polymer allow the voxels to grow together even without irradiation in the upper and lower part of the voxel. By moving the focal point following a Computer Aided Design/ Computer Aided Manufacturing (CAD/ CAM) model micro and nanostructures can be written (Selimis et al. 2015).

18.4   Applications

Even though the described processes of SL, DLP, and DLW via MPP are widely applied in technical applications for academia and industry, we restrict this overview to applications in the field of biofabrication like microfluidics, biomedical devices, tissue engineering, and drug delivery. (Pereira et al. 2015). An example for successful biofabrication is the photopolymerization of hydrogel networks that can be performed even in the presence of cells and bioactive molecules. Generation of 3D microenvironments with precise polymer architectures and varying compositions via SL and MPP provides control over cell functions by mimicking the natural environment on cellular and sub-cellular scale (Lutolf 2012; Torgersen et al. 2013). Bashir lab, for example, produced cell-laden hydrogels from PEGDA pre-polymers containing two cell types (skeletal muscle myoblasts and primary hippocampus neurons) at different locations via bottom-up stereolithography to study successfully the effects of co-encapsulation (Zorlutuna et al. 2011). Parkatzidis et al. produced grid-like structures from novel photocurable hydrogels mimicking the Extracellular Matrix (ECM) by means of MPP for 3D culturing of dental pulp stem cells (Parkatzidis, et al., 2019).
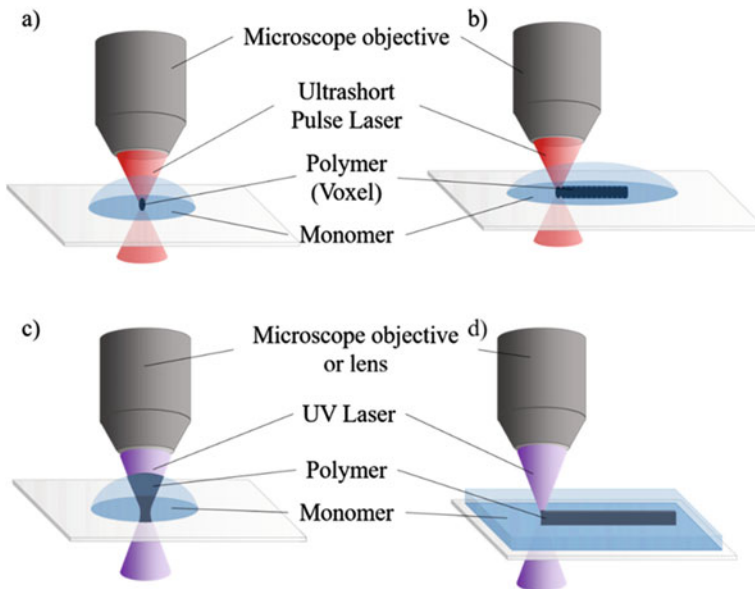
Fig. 18.2 Differences in digital laser writing via MPP and stereolithography. **a-b** DLW, **a** in a monomer solution. A polymer voxel is built in the focal plane of the ultrashort pulsed laser beam. **b** by moving the focus in the monomer volume voxels are formed next to each other and grow together by radical diffusion effects. **c-d** single photon stereolithography. **c** the monomer cross-links from the surface along the whole beam caustic according to the optical penetration depth, **d** by moving the beam over the liquid surface the monomer polymerizes in the whole monomer layer

Typical applications for single photon-based stereolithography are in the field of scaffold production for tissue engineering. The focus of these scaffolds is on the design to provide structures optimized for cell seeding and growth (Hoffmann et al. 2017) (Fig. 3a, b). Providing the right mechanical properties to allow cells to mature into a fully functional tissue. Within the human body tissue ranges, e.g., from soft and elastic fatty tissue to hard, load-bearing mandibular bone. Moreover, from relatively thin cell layers like epidermis to complex vascularized heart tissue or full-thickness skin. 3D Printing technologies allow for the systematic investigation of scaffold design. (An et al. 2015) An example is the design of branched vascular structures for full-thickness skin development, which provides optimized geometry for blood flow (Han et al. 2018) (Fig. 3c). While SL produces structures in the size of several micrometers, MPP allows the production in the range of nanometers. Nanometers correspond to the feature sizes of cell adhesion points, meaning that MPP allows for mimicking surface structure to analyze the process of cell adhesion and the mechanical load of cells on those structures (Engelhardt et al. 2011).
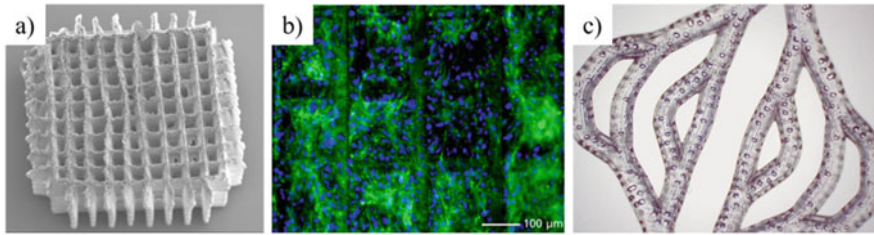
Fig. 18.3 Scaffolds produced by stereolithography: **a** Wood pile scaffold; **b** scaffold shown in **a** seeded with fibroblast cells stained with GFP and DAPI; **c** branched porous scaffold for following calculations for optimized branching angle

References

An J, Teoh JEM, Suntornnond R, Chua CK (2015) Design and 3D printing of scaffolds and tissues. Special section: advanced materials and materials. Engineering 1:261–268

Bagheri A, Jin J (2019) Photopolymerization in 3D Printing. ACS Appl Polym Mater 1:593–611. https://doi.org/10.1021/acsapm.8b00165

Chatani S, Kloxin CJ, Bowman CN (2014) The power of light in polymer science: photochemical processes to manipulate polymer formation, structure, and properties. Polym Chem 5:2187–2201. https://doi.org/10.1039/C3PY01334K

Engelhardt S, Hoch E, Borchers K, Meyer W, Krüger H, Tovar GEM, Gillner A (2011) Fabrication of 2D protein microstructures and 3D polymer–protein hybrid microstructures by two-photon polymerization. Biofabrication 3:25003. https://doi.org/10.1088/1758-5082/3/2/025003

Gauvin R, Chen Y-C, Lee JW, Soman P, Zorlutuna P, Nichol JW, Bae H, Chen S, Khademhosseini A (2012) Microfabrication of complex porous tissue engineering scaffolds using 3D projection stereolithography. Biomaterials 33:3824–3834. https://doi.org/10.1016/j.biomaterials.2012.01.048

Han X, Courseaus J, Khamassi J, Nottrodt N, Engelhardt S, Jacobsen F, Bierwisch C, Meyer W, Walter T, Weisser J, Jaeger R, Bibb R, Harris R (2018) Optimized vascular network by stereolithography for tissue engineered skin. Int J Bioprint 4:134. https://doi.org/10.18063/IJB.v4i2.134

Herzfeld KF, Göppert Mayer M (1931) Energieübertragung an adsorbierte Moleküle 1931A:669–678. https://doi.org/10.1515/zpch-1931-s172

Hoffmann A, Leonards H, Tobies N, Pongratz L, Kreuels K, Kreimendahl F, Apel C, Wehner M, Nottrodt N (2017) New stereolithographic resin providing functional surfaces for biocompatible three-dimensional printing. J Tissue Eng 8. https://doi.org/10.1177/2041731417744485

Lutolf MP (2012) Cell environments programmed with light. Nature 482:477–478. https://doi.org/10.1038/482477a

Mironov V, Trusk T, Kasyanov V, Little S, Swaja R, Markwald R (2009) Biofabrication: a 21st century manufacturing paradigm. Biofabrication 1:22001. https://doi.org/10.1088/1758-5082/1/2/022001

Miwa M, Juodkazis S, Kawakami T, Matsuo S, Misawa H (2001) Femtosecond two-photon stereolithography. Appl Phys A 73:561–566. https://doi.org/10.1007/s003390100934

Moulton P (1982) Ti-doped sapphire: tunable solid-state laser. Opt News 8(6):9–9

Ovsianikov A, Li Z, Torgersen J, Stampfl J, Liska R (2012) Selective functionalization of 3D matrices via multiphoton grafting and subsequent click chemistry. Adv Funct Mater 22:3429–3433. https://doi.org/10.1002/adfm.201200419

Parkatzidis K, Chatzinikolaidou M, Kaliva M, Bakopoulou A, Farsari M, Vamvakaki M (2019) Multiphoton 3D printing of biopolymer-based hydrogels. ACS Biomater Sci Eng 5:6161–6170. https://doi.org/10.1021/acsbiomaterials.9b01300

Pereira RF, Bártolo PJ (2015) 3D bioprinting of photocrosslinkable hydrogel constructs. J Appl Polym Sci 132. https://doi.org/10.1002/app.42458

Selimis A, Mironov V, Farsari M (2015) Direct laser writing: principles and materials for scaffold 3D printing. Microelectron Eng 132:83–89. https://doi.org/10.1016/j.mee.2014.10.001

Stampfl J, Baudis S, Heller C, Liska R, Neumeister A, Kling R, Ostendorf A, Spitzbart M (2008) Photopolymers with tunable mechanical properties processed by laser-based high-resolution stereolithography. J Micromech Microeng 18:125014. https://doi.org/10.1088/0960-1317/18/12/125014

Stokker-Cheregi F, Palla-Papavlu A, Paun IA, Lippert T, Dinescu M (2018) Laser structuring of soft materials: laser-induced forward transfer and two-photon polymerization. In: Ossi PM (ed) Advances in the application of lasers in materials science. Springer International Publishing, Cham, pp 247–273

Torgersen J, Qin X-H, Li Z, Ovsianikov A, Liska R, Stampfl J (2013) Hydrogels for two-photon polymerization: a toolbox for mimicking the extracellular matrix. Adv Funct Mater 23:4542–4554. https://doi.org/10.1002/adfm.201203880

Whitby R, Ben-Tal Y, MacMillan R, Janssens S, Raymond S, Clarke D, Jin J, Kay A, Simpson MC (2017) Photoinitiators for two-photon polymerisation: effect of branching and viscosity on polymerisation thresholds. RSC Adv 7:13232–13239. https://doi.org/10.1039/C6RA27176F

Zorlutuna P, Jeong JH, Kong H, Bashir R (19/2011) Tissue engineering: stereolithography-based hydrogel microenvironments to examine cellular interactions. Adv Funct Mater 21:3597. https://doi.org/10.1002/adfm.201190080