A review on stereolithography and its applications in biomedical engineering

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Abstract

Stereolithography is a solid freeform technique (SFF) that was introduced in the late 1980s. Although many other techniques have been developed since then, stereolithography remains one of the most powerful and versatile of all SFF techniques. It has the highest fabrication accuracy and an increasing number of materials that can be processed is becoming available. In this paper we discuss the characteristic features of the stereolithography technique and compare it to other SFF techniques. The biomedical applications of stereolithography are reviewed, as well as the biodegradable resin materials that have been developed for use with stereolithography. Finally, an overview of the application of stereolithography in preparing porous structures for tissue engineering is given.

Introduction

The advance of solid freeform fabrication techniques has significantly improved the ability to prepare structures with precise geometries, using computer aided designs and data from (medical) imaging [1]. These techniques include selective laser sintering, fused deposition modelling, 3D printing and stereolithography. Stereolithography is particularly versatile with respect to the freedom of designing structures and the scales at which these can be built: sub-micron sized structures as well as decimetre-sized objects have been fabricated. In the biomedical field, these developments have led to the fabrication of patient-specific models for mould-assisted implant fabrication [2], aids for complex surgery [3] and tailor-made parts such as hearing aids. More recently, biodegradable materials have been developed for the preparation of medical implants, such as tissue engineering scaffolds, by stereolithography [4-12].

In this paper we review the materials that have been developed for stereolithography, and their use in the biomedical field. The principles of operation of the technique are discussed. The chemistry, mechanical properties and degradation behaviour of structures built by stereolithography are considered, especially with regard to their application as an implantable device.

Rapid prototyping and manufacturing

Originally, solid freeform fabrication techniques were developed to create prototypes for purposes of designing new products. Traditional prototyping methods involve laborious mould making and casting steps [13], whereas the ability to create an object within hours from a computer design by rapid prototyping (RP) significantly speeds up the development of products. Currently, rapid prototyping using SFF techniques is common practice in the automotive industry, for jewellery making and for designing end-user devices and appliances [14]. Also in designing surgical tools, implants and other biomedical devices, these additive fabrication methods have been used.

As solid freeform fabrication technologies are continuously evolving, fabrication costs are decreasing and the properties of the manufactured parts are becoming better. Therefore, these techniques are more and more being used for the rapid manufacturing of products in small series. The time gain in product development, freedom of design and tool-free fabrication can outweigh the increased fabrication costs per item [14].

Stereolithography was developed by 3D Systems in 1986, being the first commercially available SFF technique. Several other techniques have been developed over the past 20 years; **Fig. 1** gives an overview of the major SFF techniques employed for biomedical applications. Different setups are required to build objects of different sizes, but each technique has a lower limit in size of the smallest details that can be produced. In general, there is a clear relationship between the scale at which an object can be built by an SFF technique and the resolution with which it is built: the higher the resolution with which a part can be built, the smaller will be its maximum size.

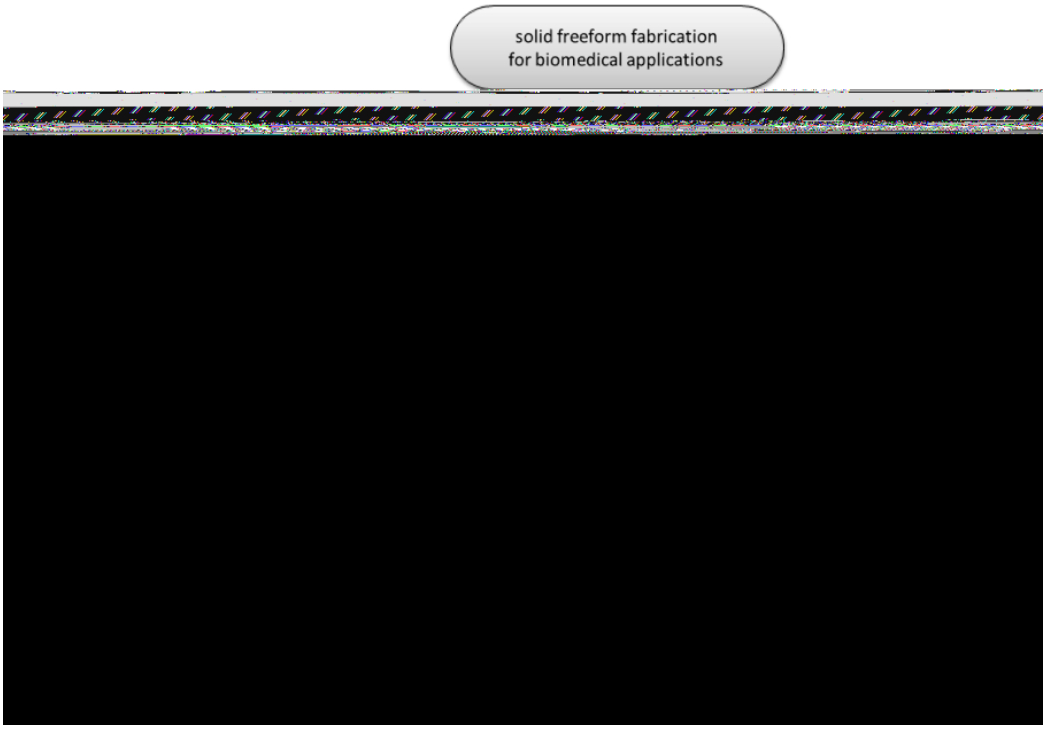

Fig. 1. Overview of additive processing/solid freeform fabrication technologies applied for biomedical applications. For each technique, the symbols indicate whether polymers and/or ceramics, hydrogels, and living cells have been employed.

Regarding accuracy and resolution, stereolithography is superior to all other SFF techniques. While in most fabrication techniques the smallest details are 50-200 μm in size, many commercially available stereolithography setups can build objects that measure several cubic centimetres at an accuracy of 20 μm. Stereolithography setups have been developed that make use of two-photon initiation of the polymerisation reaction, and in the laboratory micron-sized structures with sub-micron resolution have been fabricated using these setups [15]. This accuracy has not been achieved with other RP techniques.

Stereolithography

Like most solid freeform fabrication techniques, stereolithography is an additive fabrication process that allows the fabrication of parts from a computer-aided design (CAD) file. The designed external and internal (pore) geometry of the structure that is to be built can either be devised using 3D drawing computer software, be described using mathematical equations [16], or be derived from scanning data of (clinical) imaging

technologies such as magnetic resonance imaging (MRI), or tomography techniques [17]. The possibility to use data from scans make these manufacturing technologies particularly useful for many applications in biomedical engineering, as it enables to fabricate patient-specific models or implants. The CAD-file describes the geometry and size of the parts to be built. For this, the STL file format was developed; an STL file lists the coordinates of triangles that together make up the surface of the designed 3D structure. This designed structure is (virtually) sliced into layers of the thickness that is used in the layer-by-layer fabrication process (usually in the range of 25-100 μm). These data are then uploaded to the stereolithography apparatus (SLA) and the structure is fabricated (**Fig. 2**). Computed tomography (CT)-scanning of the built structures allows assessing the accuracy of the process, by comparing the scan data to the design.

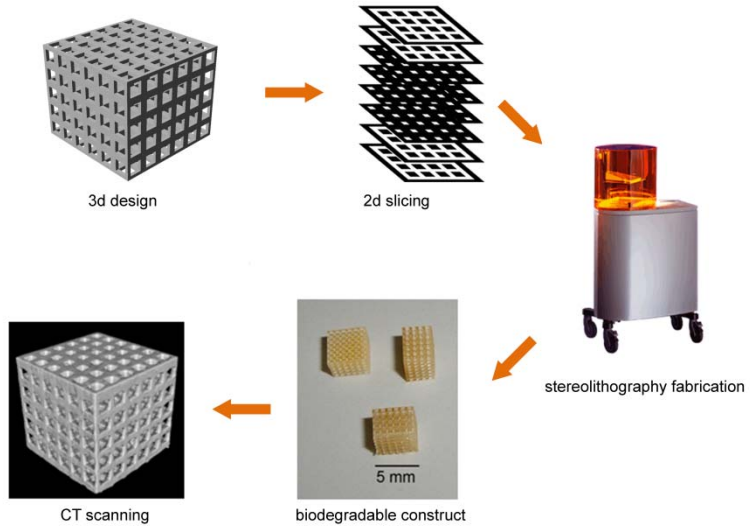

Fig. 2. Overview of the processes involved in the design and fabrication of structures by stereolithography.

The manufacturing of 3D objects by stereolithography is based on the spatially controlled solidification of a liquid resin by photo-polymerisation. Using a computer-controlled laser beam or a digital light projector with a computer-driven building stage, a pattern is illuminated on the surface of a resin. As a result of this, the resin in the pattern is solidified to a defined depth, causing it to adhere to a support platform. After photo-polymerisation of the first layer, the platform is moved away from the surface and the built layer is recoated with liquid resin. A pattern is then cured in this second layer. As the depth of curing is slightly larger than the

platform step height, good adherence to the first layer is ensured (unreacted functional groups on the solidified structure in the first layer polymerise with the illuminated resin in the second layer).

These steps (the movement of the platform and the curing of an individual pattern in a layer of resin) are repeated to construct a solid, three-dimensional object. After draining and washing-off excess resin, an as-fabricated (or green) structure is obtained. In this structure, the conversion of reactive groups is usually incomplete, and post-curing with (stroboscopic) ultraviolet light is often done to improve mechanical properties of the structures.

Fig. 3 shows schematic diagrams of two types of stereolithography setups. In both systems objects are built in a layer-by-layer manner by spatially-controlled photo-polymerisation of a liquid resin; differences are in the build orientation and in the method of illumination.

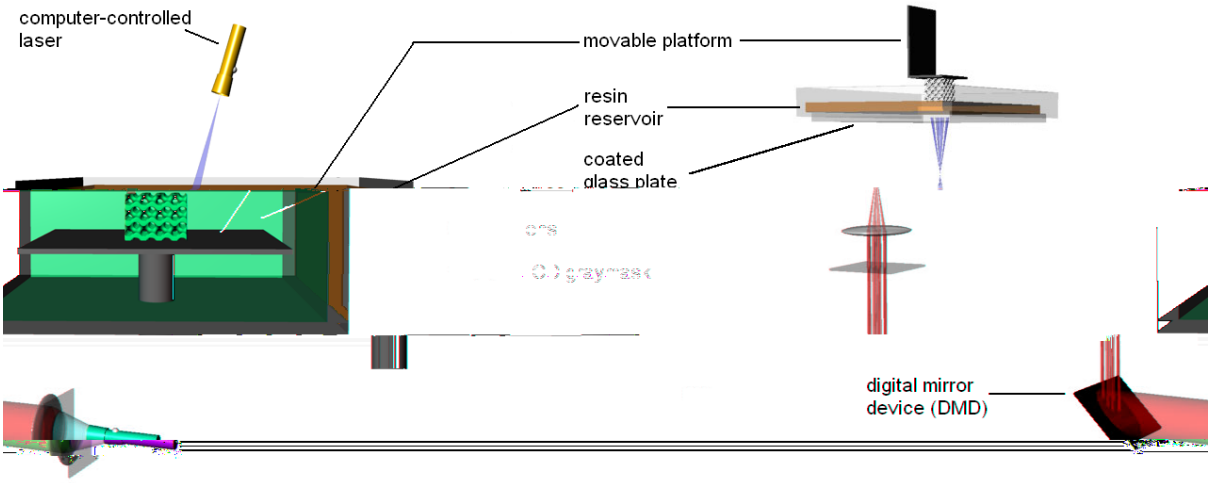

Fig. 3. Schemes of two types of stereolithography setups. Left: a bottom-up system with scanning laser. Right: a top-down setup with digital light projection.

To date, most SLA setups in use resemble the ones first developed [4, 6, 7, 18]. Using a computer-controlled laser beam to draw a pattern, structures are built bottom-up from a support platform that rests just below the resin surface (**Fig.3** left). Only a thin layer of resin is illuminated from above, and cured on top of the structure as it is built in a layer-by-layer manner. A top-down approach is increasingly being applied in stereolithography. In such setups, light is projected on a transparent, non-adhering plate from underneath (the transparent plate

forms the bottom of the vessel that contains the resin), and the support or build platform is dipped into the resin from above (**Fig. 3** right). Although the structures are subjected to larger mechanical forces, as they have to be separated from the bottom plate after illumination of each layer, this approach has several advantages over the bottom-up systems: recoating of the structure is not required, the surface being illuminated is always smooth, only small amounts of resin are required, and the illuminated layer is not exposed to the atmosphere, so oxygen inhibition is limited.

Digital light projection (DLP) is emerging as a method of illuminating the resin [11, 12, 19-21]. In this technology a digital mirror device (DMD), an array of up to several millions of mirrors that can be rotated independently to an on- and off state, is used. By projecting a two-dimensional pixel-pattern onto the transparent plate, a complete layer of resin can be cured at once (**Fig. 3** right). Build times are much reduced, as they only depend on the layer thickness and on the required exposure time, and not on their size in the xy-plane or on the number of structures being built simultaneously.

In stereolithography, control of the thickness of the layer that is cured is essential. For a given resin, the cure depth is determined by the energy of the light to which the resin is exposed. This energy can be controlled by adjusting the power of the light source, and the scanning speed (for laser systems) or the exposure time (for projection systems). The kinetics of the curing reactions taking place are quite complex. Although the different stages of the addition-type polymerisation (initiation-propagation-termination) can be expressed mathematically, the presence of multifunctional monomers and the transition of the polymerising liquid to a solid make its description more complicated. The kinetics of photo-initiated multi-vinyl polymerisations have been discussed in an extensive review by Andrzejewska [22].

In practice, much simpler equations are used to describe the polymerisation kinetics in the fabrication of structures by stereolithography. A semi-empirical equation that relates the thickness of a solidified layer (the cure depth, C_d in μm) to the light irradiation dose E (mJ/cm^2) is used: $C_d = D_p \ln (E / E_c)$. A plot of the determined cure depth (or cured layer thickness) versus the applied irradiation dose is termed a working curve [23], and is constructed to determine the correct settings for stereolithography fabrication. This equation is an adapted form of the Beer-Lambert equation, which describes the exponential decay of the intensity of light as it passes through a medium in which it is absorbed. In photo-polymerisations, the time required to reach the

gel point depends linearly on the intensity of the light at that specific location. Therefore, the depth at which the resin is cured to the gel point (C_d) increases logarithmically with time, and thus with the applied irradiation dose (E). For a specific stereolithography setup, a resin can be characterised by a critical energy E_c (mJ/cm^2) and a penetration depth D_p (μm). As the applied irradiation dose (E) exceeds the critical energy required to reach the gel point (E_c), a solidified layer forms from the resin surface. The value of E_c depends, among others, on the concentrations of photo-initiator, and of dissolved oxygen and other inhibiting species. The penetration of light into the resin is directly related to the extinction coefficient in the Beer-Lambert equation, and is characterised by D_p.

To ensure chemical and mechanical bonding between the layers during building, the macromer conversion at the interface between layers should be slightly higher than the gel point. However, this overexposure results in (further) curing into the preceding layer, and volume elements in the preceding layer that according to the design were intended to remain uncured, will now have partially polymerised. Particularly when preparing porous structures, the effect of over-cure can be significant. A high extinction coefficient of the resin corresponds to a low light penetration depth (D_p), and will allow most accurate control of the polymerisation process and minimal over-cure. The penetration depth can be decreased by increasing the photo-initiator concentration, or by including a dye in the resin. This non-reactive component competes with the photo-initiator in absorbing light. Although particularly useful when visible light sources are employed (as in general photo-initiators have low extinction coefficients in this range), the use of UV absorbers is also reported [24]. It should be realised that decreasing the light penetration depth will lead to increased building times.

Resins used in stereolithography

The limited number of resins that are commercially available for processing by stereolithography has often been considered the main limitation of the technique. The resin should be a liquid that rapidly solidifies upon illumination with light. The first resins developed for use in stereolithography were based on low-molecular weight polyacrylate or –epoxy macromers that form glassy networks upon photo-initiated polymerisation and crosslinking. Several resins have been developed over the past two decades, and the mechanical properties of the networks obtained after curing cover a wide range. The properties of parts built by stereolithography are continuously improving, making them not only useful as prototypes but also as functional parts for more

demanding end-use applications [25]. Resins that can be used to create biodegradable devices for application in medicine are being developed as well, see below.

Most of the available stereolithography resins are based on low-molecular weight, multi-functional monomers, and highly crosslinked networks are formed. These materials are predominantly glassy, rigid and brittle. Only few resins have been described that allow the preparation of elastomeric objects by stereolithography. These resin formulations include macromers with low glass transition temperatures and relatively high molecular weights (1-5 kg/mol), often in combination with non-reactive diluents such as N-methylpyrrolidone (NMP) or water to reduce the viscosity of the resin [26-28].

To create polymer-ceramic composite objects [29-32], ceramic particles (*e.g.* alumina or hydroxyapatite) are homogeneously suspended in the stereolithography resin and photo-polymerised in the SLA. Processing of the resin is more difficult, as the viscosity of the resin can significantly increase upon addition of the powder. Maximum ceramic contents of up to 53 wt% have been reported [33]. Furthermore, the ceramic particle size should be smaller than the layer thickness in the building process to prepare the objects accurately. The fabricated composite structures are in general, stiffer and stronger than the polymeric structures. Starting from these composite structures, all-ceramic objects have been made by first fabricating a composite structure by stereolithography and then burning out the polymer (pyrolysis) and sintering the ceramic particles [33-35].

Different resins have been processed using stereolithography, leading to objects with widely differing characteristics. Although the number of resins that is available continues to increase, the technique is still limited to the use of a single resin at a time. (Note that 3D printing- and plotting techniques related to fused deposition modelling allow the use of multiple cartridges to prepare structures using different materials simultaneously.) The ability to pattern multiple resins in a construct (and even within a single layer) is possible in stereolithography too, but complex sequential polymerisation and rinsing steps are required for each layer built [28, 36]. A major technological challenge lies in developing an automated system to remove uncured resin and exchange resin reservoirs. The restriction to use one resin in stereolithography is perhaps the true major limitation of the technique.

Applications of stereolithography in biomedical engineering

The possibilities for using stereolithographic fabrication methods for biomedical applications are numerous. But, despite that the technique has been commercially available for more than 20 years; it is still not extensively used in the medical field. Below, an overview is given of (possible) biomedical applications of stereolithography.

Patient-specific models and functional parts

The ability to use data from (clinical) imaging techniques like MRI or CT makes stereolithography particularly useful for biomedical applications. Initially, these images were only used for diagnosis and pre-operative planning. With the progress in computer-aided design and manufacturing technologies, it is now possible to make use of this information in conducting the surgery itself: by making use of patient-specific models of parts of the body fabricated by stereolithography, the time in the operating theatre and the risks involved can significantly be reduced [37, 38]. In implantations using drill guides built by stereolithography, the accuracy of the implantation was much improved when compared to using conventional surgical guides, particularly in oral surgery [3, 39]. Stereolithography has been also used to fabricate moulds for the preparation of implants in cranial surgery, using data from CT scans [2, 40].

In the abovementioned cases, the function of the structures prepared by stereolithography is in aiding the surgeon. Furthermore, the combination of medical imaging and stereolithography has been used to make models or moulds for preparing anatomically shaped implants. In this way, Sodian *et al*. prepared customised heart valves that could be placed without the need for suturing [41]. Other studies on such use of computer-aided fabrication are ear-shaped implants [42] and aortas [43]. Other tailored parts, such as hearing aids, have specific functionalities as well and are applied in contact with the body; they are now routinely manufactured using stereolithography. These applications illustrate the benefits of stereolithography in the manufacturing of tailored parts for use in the clinical practice.

Implantable devices

Besides for the fabrication of surgical models, stereolithography can be used to fabricate patient-specific implantable devices. In trauma surgery, for example, a tailored implant for facial reconstruction can be obtained by imaging the undamaged side of the head and reproducing its mirror image by stereolithography. Also, a CT-image of bone with a defect due to the removal of a tumour could be used to prepare a custom-fit, biodegradable implant that supports the regeneration of bone.

For prototyping, the appearance of the built parts, its mechanical properties and biocompatibility are not very important, and most commercially available resins suffice for this purpose. However, for the direct fabrication of implantable devices, these properties are of utmost importance and special resins need to be developed and used.

The implantation of devices prepared by stereolithography has only been reported in a few cases. Matsuda *et al.* showed that degradable crosslinked structures prepared by stereolithography using poly(trimethylene carbonate-*co*-ε-caprolactone) resins caused no adverse effects after a 1 month implantation period under the dorsal skin of rats [8]. Popov *et al.* prepared non-resorbable polyacrylate and hydroxyapatite composite parts, and implanted them into the femurs of rats for time periods of up to 8 weeks [31]. Although many crosslinked polymers are not cytotoxic, the unreacted monomer and photo-initiator residues trapped in the densely crosslinked networks can be. For this reason, the built parts were extracted before the implantation using supercritical CO_2. It was shown that the extracted composite implants integrated well with surrounding bone, and that new bone had formed at the surface of the implants. This shows that anatomically shaped implants, compatible with cells and surrounding tissues can be manufactured using stereolithography.

Numerous other medical implants with tailored geometries and physical properties, such as bone fracture fixation devices, parts for artificial hips or knees, nerve guidance channels or prostheses, can be manufactured by stereolithography. Liska and co-workers characterised an extensive library of photo-curable non-cytotoxic resin materials with a wide range of physical and chemical properties that could be used for these long-term applications (**Fig. 4**) [21, 44, 45].

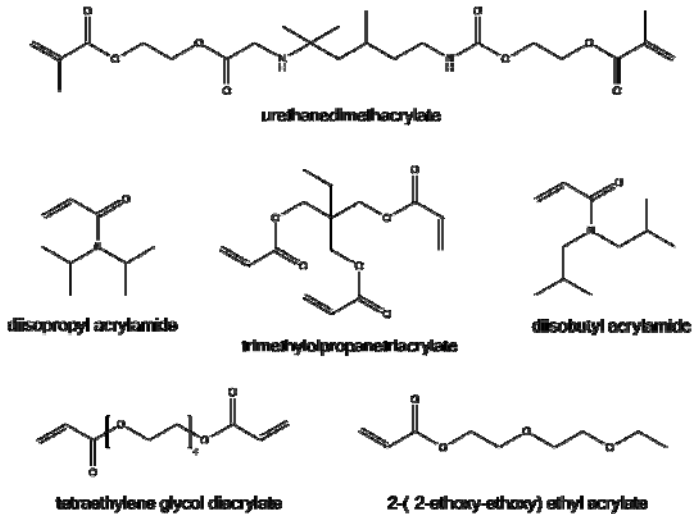

Fig. 4. Biocompatible macromers for use in the formulation of resins for stereolithographic fabrication of durable implants [21].

Tissue engineering

Tissue engineering is a field in biomedical engineering that is developing fast. In tissue engineering, a resorbable scaffolding structure is used in combination with cells and/or biologically active compounds to induce the (re)generation of tissues *in vitro* or *in vivo* [46]. The scaffold is a porous implant, intended as a temporary support structure for seeded cells and formed tissues. Upon implantation, it should not elicit severe inflammatory responses, and it should degrade into non-toxic compounds as newly formed tissue is formed. The scaffold is a template that allows cell adhesion and directs the formation of tissue, therefore the architecture of its inner pore network and its outer geometry need to be well-defined. For this reason, solid freeform fabrication techniques play an important role in the manufacturing of advanced tissue engineering scaffolds. The fabrication of precisely defined tissue engineering scaffolds by stereolithography is becoming a new standard. Much work has been done in developing biodegradable macromers and resins. In 2000, a first report on the preparation of biodegradable structures by stereolithography appeared [9]. Liquid low molecular weight copolymers of ε-caprolactone and trimethylene carbonate were prepared by ring opening polymerisation using a polyol as initiator, and subsequently derivated at the hydroxyl termini with coumarin. Later, the same group published the use of similar oligomers, but now end-functionalised with methacrylate groups [8]. **Fig. 5** shows the structures fabricated using these macromers.

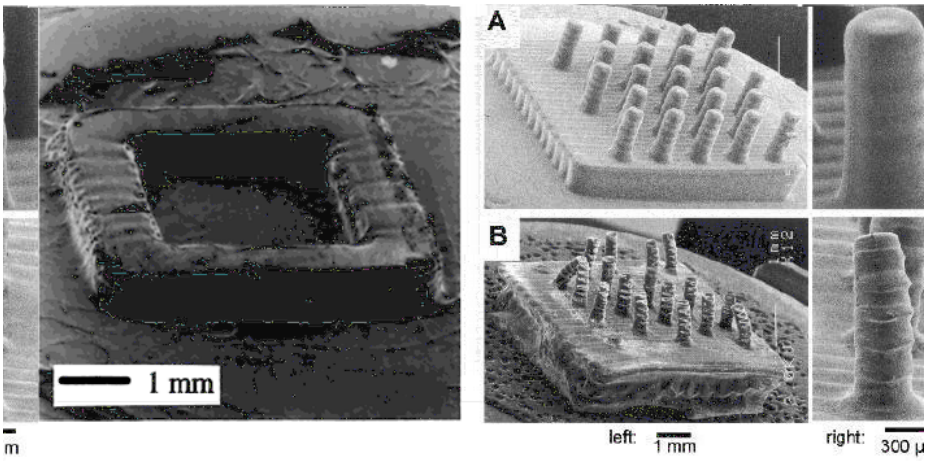

Fig. 5. SEM images of biodegradable structures built by stereolithography intended for application in tissue engineering. Left: structures prepared from P(TMC-*co*-CL)-coumarin macromers [9]. Right: structure prepared from P(TMC-*co*-CL)-acrylate (A) before and (B) after 1 month of implantation [8].

The biodegradable macromers that have been applied in stereolithography are based on functionalised oligomers with hydrolysable ester- or carbonate linkages in the main chain. Among these macromers are those based on poly(propylene fumarate) (PPF) [4], trimethylene carbonate (TMC) and ε-caprolactone (CL) [8-10], or D,L-lactide (DLLA) [5, 12] (**Fig. 6**).

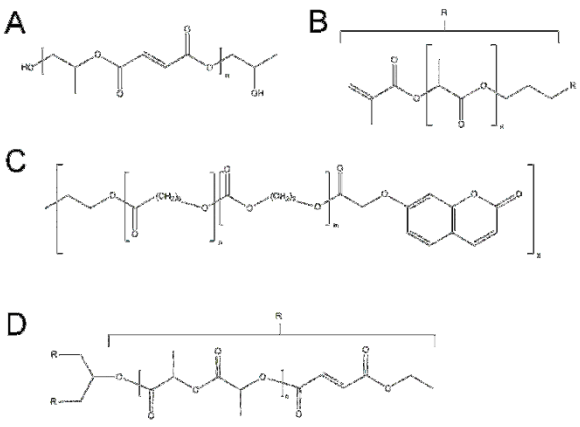

Fig. 6. Examples of biodegradable macromers used in stereolithography. A. Polypropylene fumarate (PPF) [4, 6, 67]. B. Linear poly(D,L-lactide)-methacrylate [12]. C. Poly(TMC-co-CL)-coumarin [68]. D. Star-shaped poly(D,L-lactide)-fumarate [5].

The viscosity of low molecular weight macromers that have a low glass transition temperature (such as those based on TMC and CL) can be sufficiently low to allow direct processing in the stereolithography apparatus (although it should be noted that this can result in crosslinked structures with relatively poor mechanical properties). For macromers of higher molecular weights, or with relatively high glass transition temperatures (such as those based on DLLA or PPF) a diluent is required to reduce the viscosity of the resin. Typically, the highest resin viscosities that can be employed in stereolithography are approximately 5 Pa·s [33]. Macromers that contain fumarate (end)groups require a reactive diluent such as diethyl fumarate [6] or N-vinyl-2-pyrrolidone [5] to reach appropriate reaction rates. Solid or highly viscous macromers that are functionalised with more reactive groups such as (meth)acrylates, can also be diluted with non-reactive diluents. In this case, the cured material shrinks upon extraction of the diluent. As this shrinkage is isotropic, it can be taken into account when designing the scaffold structures [12]. The stereolithography resins summarised above have enabled the fabrication of biodegradable tissue engineering scaffolds with a range of properties. **Fig. 7** gives two examples of tissue engineering scaffolds with well-defined architectures built by stereolithography, the size scales are relevant for tissue engineering. Scaffolds with hexagonal and cubic pores (both prepared from a PPF/DEF resin) and scaffolds with gyroid pore network architecture (PDLLA-fumarate/NVP resin) are depicted in the figure.

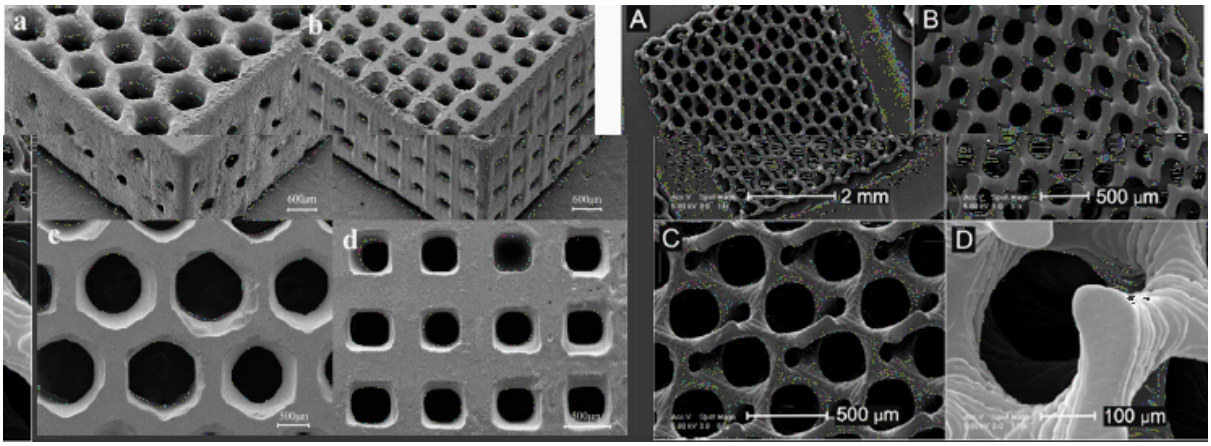

Fig. 7. Examples of tissue engineering scaffolds built by stereolithography using PPF or PDLLA-fumarate and a reactive diluent. Left: structures prepared from PPF and DEF as a reactive diluent [6]. Right: structures prepared from PDLLA-fumarate and NVP as a reactive diluent [5].

The scaffold materials presented above have shown to support cell adhesion and growth. In general however, the interaction between cells and synthetic polymers is not very strong, and adhesion of the cells is facilitated by the adsorption of proteins (from the culture medium) on the surface of the pores of the scaffold. Alternatively, bioactive compounds can be included in the scaffold fabrication process. To improve the bioactivity of scaffolds in bone tissue engineering, for example, composite structures containing hydroxyapatite have been fabricated using resins containing dispersed hydroxyapatite particles. By mixing PPF and hydroxyapatite particles in diethyl fumarate as reactive diluent, a photo-polymerisable composite resin was obtained [29]. Furthermore, specific protein-binding molecules have been included in the resins [47], and proteins have been grafted onto the surface of the scaffold network [48]. Most promising are recent developments in the functionalisation of natural polymers. For the first time, using methacrylate-functionalised gelatin, an object has been made by stereolithography from a natural polymer [49]. Before, porous structures prepared by photo-curing modified chitosan showed very good endochondral ossification upon implantation [50]. Other natural polymers that have been modified to enable photo-curing are (meth)acrylated oligopeptides [51] and methacrylated hyaluronic acid [52]. It is likely that these materials will be used in stereolithography in the near future.

Stereolithography requires the use of a liquid photo-sensitive formulation that solidifies upon illumination. In general, photo-initiated addition polymerisations lead to stable non-degradable polymer chains. For application in tissue engineering, it is important that degradation products of the material from which the scaffold is prepared do not accumulate in the body. This is also of importance in other areas where degradable implants are used. The molecular weight and character of the remaining addition-type polymer determines if it can be excreted by renal clearance [53]. The kinetic chain length strongly depends on the initiating conditions of the polymerisation (nature and concentration of the photo-initiator, light intensity), and has been determined after degradation of the network [54-56]. Widely ranging values, corresponding to several monomer repeat units [55] or to several thousands of monomer repeat units [54] have been reported. When employing light-induced dimerisation reactions, such as has been done with the photo-polymerisation of macromers functionalised with coumarin [9] or phenyl azide [57] end-groups, long kinetic chains are not formed.

Stereolithography enables the reproducible manufacturing of tissue engineering scaffolds with well-defined architectures. However, only few attempts have been made to investigate the influence of the scaffold architecture on cell culture and tissue formation. Chu *et al.* used stereolithography to prepare hydroxyapatite scaffolds with two different pore network architectures, and investigated the regeneration of bone in the mandibles of Yucatan minipigs [35]. Significant differences between the different scaffold designs were observed with regard to the amount, distribution and functionality of the generated tissue. This indicates the importance of the pore architecture of the scaffold in *in vivo* bone formation, although extensive research is needed to draw general conclusions.

Using the various resins developed for use in stereolithography, biodegradable scaffolds have been manufactured. Although these scaffolds proved to support cell attachment *in vitro* or to cause no adverse effects *in vivo*, the major advantage of using stereolithography, being able to prepare well-defined designed architectures, has not yet been fully utilised. Scaffolds that have been designed to have optimal mechanical and cell-delivery properties for specific applications can now be fabricated, allowing to verify the numerical models [58]. The effect of scaffold (pore network) architecture on tissue (re)generation can be investigated in detail. In the regeneration of bone for example, many different values for the optimal pore size in bone regeneration have been reported. In these studies comparisons are made in which not only the average pore size of the scaffolds differs, but different materials with different pore network architectures are employed under different experimental conditions [59-61].

Cell-containing hydrogels

By encapsulating cells in fabricated structures, higher cell densities might be achieved than by seeding into built porous scaffolds. Also, the distribution of cells might be better controlled. Various studies have reported the use of water-soluble di(meth)acrylated poly(ethylene glycol) (PEG-DMA) to create structured, cell-containing hydrogels by stereolithography. Dhariwala *et al.* were the first to successfully encapsulate (Chinese hamster ovary) cells in PEG-DMA hydrogels, using stereolithography [27]. Later, PEG-DMA constructs containing encapsulated human dermal fibroblasts (**Fig. 8**) [36], and PEG-diacrylate gel structures containing marrow stromal cells [11] were reported. In these cases, large numbers of cells could be encapsulated at high densities (several millions of cells per mL). The cells showed good survival after fabrication of the construct, especially when peptides containing RGD-sequences were incorporated in the gels [36].

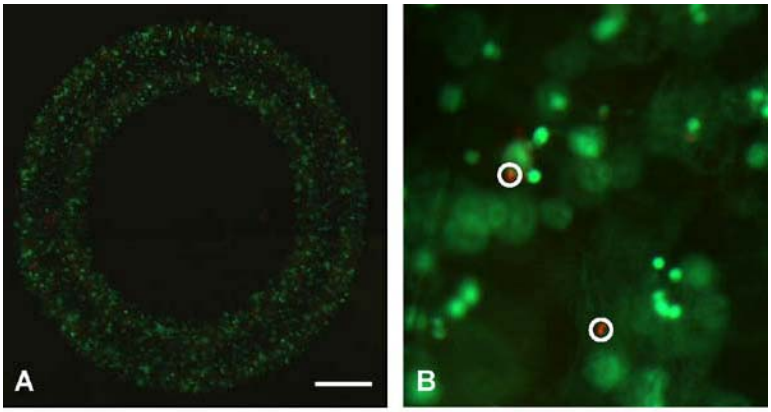

Fig. 8. Poly(ethylene glycol)-dimethacrylate hydrogels with encapsulated cells prepared by stereolithography [36]. Live/Dead assay on human dermal fibroblasts encapsulated in the gel. Scalebar represents 1 mm.

In the given examples, the cell-containing structures that were prepared by stereolithography were very elementary and consisted of only a few built layers. To create more complex 3D structures, multiple layers and longer build times would have been required. The least cytotoxic of known photo-initiators, Irgacure 2959 (2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone) [62], is cytotoxic at the concentrations necessary: cell survival is approximately 25 % after 24 h in an aqueous PEG-DMA resin containing 0.5 % w/v Irgacure 2959 [36]. The limited availability of cells and the possibility of non-homogeneous cell distributions due to settling of the cells in time could be other reasons why more complex structures were not prepared. With elastic modulus values of approximately 1 kPa [27], these hydrogels seem well suited for the engineering of soft tissues, although it seems clear that much more research will need to be conducted in this direction.

New developments in stereolithography and related technologies

Two-photon polymerisation is increasingly used in stereolithographic fabrication. In two-photon polymerisation, the photo-initiator is excited by the (nearly) simultaneous absorption of two photons with relatively low intensity, which together introduce enough energy to break the labile bond and initiate the polymerisation reaction. As a result, two-photon polymerisation is a non-linear optical process in which the polymerisation rate is proportional to the square of the laser intensity, as opposed to a linear relationship as is

the case for single-photon polymerisations. This leads to a more localised initiation of the polymerisation, and therefore to higher resolutions. Using stereolithography setups based on two-photon absorption, resolutions as high as 200 nm can be obtained. A review on two-photon polymerisation was published by Lee *et al* [63], and first applications in tissue engineering have also been reported [64].

Holography or interference lithography is a technique in which two or more light sources are used to create an interference pattern. By superposition of the light waves, regular patterns with locally varying light intensities are obtained [65]. It is a well-known process for the creation of micro- and nanostructures like nanopillars, nanostructured substrates, microframes, 3D photonic crystals and microsieves [66]. Interference holography provides a faster and more accurate method to solidify patterns in a photo-curable resin than can be achieved with stereolithography, and although it is restricted to a limited number of patterns it could be of interest to prepare repetitive porous structures for tissue engineering.

Conclusions

Stereolithography is a solid freeform fabrication technique that is particularly versatile with respect to the freedom of design of the structures that are to be built, and to the scales at which these can be built. It has a strong prospective for biomedical applications, especially in combination with medical imaging techniques such as MRI and CT. It has proven to facilitate, speed up, and improve the quality of surgical procedures such as implant placements and complex surgeries. Also, anatomically-shaped implants and tailor-made biomedical devices, such as hearing aids, have been prepared using stereolithography. The development of new resins has enabled to directly fabricate implantable devices like biodegradable tissue engineering scaffolds. With the introduction of hydroxyapatite composites, peptide-grafted structures, cell-containing hydrogels and modified natural polymers, stereolithography has developed into a broadly applicable technique for biomedical engineering purposes.

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