Polymer based scaffolds for tissue regeneration by stereolithography

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Stereolithography is a rapid prototyping technique, introduced in the 1980s, that enables the realization of complex 3D structures for tissue engineering directly from a computer model. Although many other 3D printing techniques have been developed over the last decades, it has the highest fabrication accuracy and an increasing number of materials that can be processed is becoming available. In this review we present the characteristic features of the stereolithography technique and the design of polymers (of both synthetic and natural origin) with tailored structure, architecture, and functionality for stereolithography to mimic a broad range of human tissues. In addition, the use of two-photon polymerization for the production of tissue engineering scaffolds with smaller-scale features than those typical of the conventional stereolithography technology is described.

Keywords: stereolithography, rapid prototyping, biomaterials, tissue engineering, scaffolds

1. Introduction

The fundamental concept underlying tissue engineering is to combine a scaffold or matrix, with living cells, and/or biologically active molecules to form a tissue engineering construct (TEC) to promote the repair and/or regeneration of tissues [1]. The scaffold attempts to mimic the function of the natural extracellular matrix. A successful scaffold should meet some basic requirements: (a) to serve as an adhesion substrate for the cell, facilitating the localization and delivery of cells when they are implanted; (b) to provide temporary mechanical support to the newly grown tissue by defining and maintaining a 3D structure; and (c) to guide the development of new tissues with the appropriate function [2–4]. Further, for their design physicochemical properties, morphology and degradation kinetics need to be considered. The external size and shape of the construct are of importance, particularly if it is customized for an individual patient [5]. Depending on scaffolding material and TE strategy, different processing techniques and methodologies have been proposed to optimize final scaffold performances in terms of external shape and size, surface morphology and internal architecture. These include, among others, solvent casting combined with particulate leaching, freeze drying, gas foaming, melt moulding, fibre bonding, phase separation techniques, electrospinning and additive manufacturing (AM) techniques [6, 7]. In recent years, a number of automated fabrication methods have been employed to create scaffolds with well-defined architectures [8, 9]. According to the latest ASTM standards these have been classified as additive manufacturing (AM) techniques or as rapid prototyping (RP) techniques [10]. RP is a common name for a group of techniques that can generate a physical model directly from computer-aided design data (CAD). Unlike conventional machining, which involves constant removal of materials, RP builds parts by selectively adding materials layer by layer, as specified by a computer program, where each layer represents the shape of the cross-section of the model at a specific level [11]. Rapid prototyping is defined as the process of joining materials to make objects from three-dimensional (3D) model data and it has been extensively applied for the fabrication of TE scaffolds by means of different techniques [7], such as stereolithography (SLA) [12, 13] and fused deposition modelling (FDM) [9, 14]. These techniques enable the fabrication of 3D structures with a predefined geometry and size, and with a porous architecture characterized by a fully interconnected network of pores with customizable size, shape and distribution [15, 16]. Originally RP techniques were developed to create

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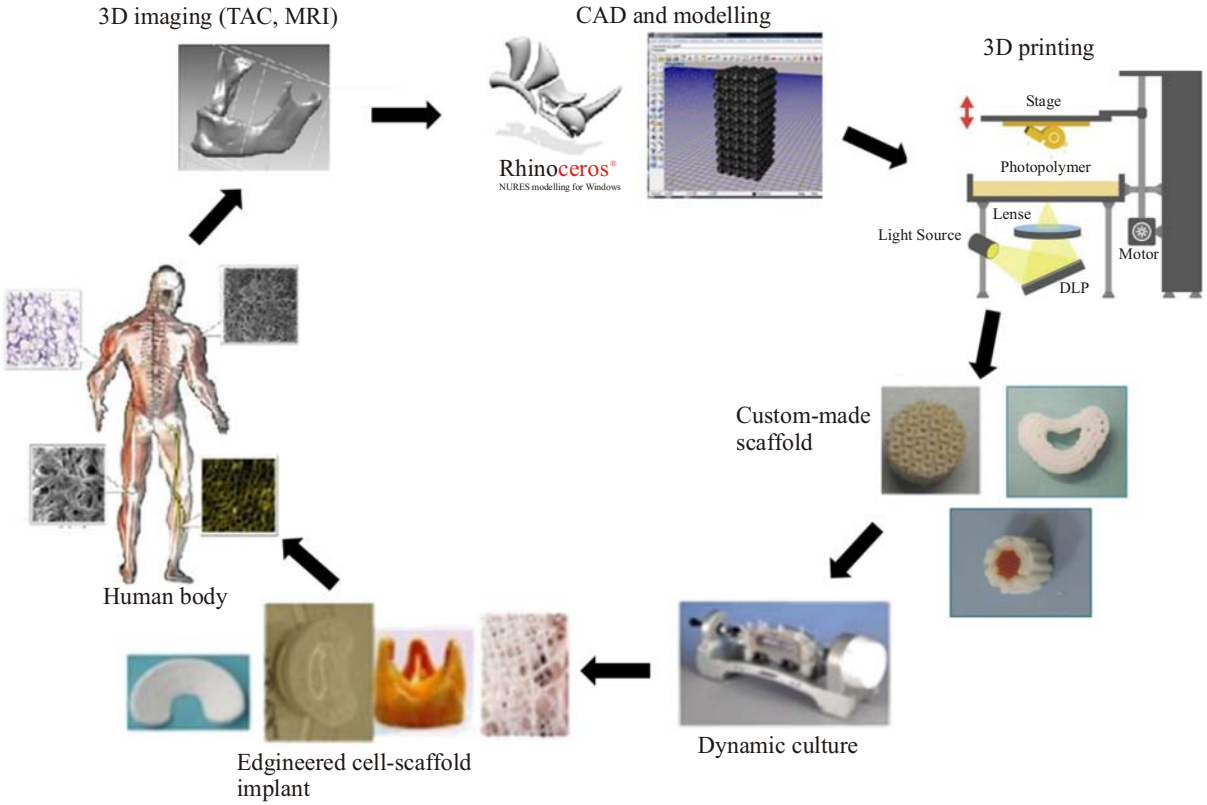

Fig. 1. Schematic overview of the additive manufacturing process applied to tissue engineering.

prototypes for purposes of designing new products, but due to the ability to create an object within hours from a CAD design, RP has become a common practice in the automotive industry, for jewellery making and for designing end-user devices and appliances [17]. These additive fabrication methods are also being used in designing surgical tools, implants and other biomedical devices. With respect to medical implants, patients might have individual needs, based on specific anatomy or the possibility to include autologous cells to enhance the treatment. The combination of automation and flexibility in design is what makes RP very suitable for the generation of such personalized implants and devices [18, 19]. 3D model data for scaffold development can be derived from medical imaging techniques used for diagnostic purposes, such as computer tomography (CT) and magnetic resonance imaging (MRI), and are generally treated by computer-aided design (CAD) (Fig. 1) [20, 21].

Alternatively a simplified 3D model can be directly designed in CAD software or developed by means of mathematical equations [22, 23] or topological optimization [24]. Depending on the fabrication principle, the most extensively applied RP techniques for tissue engineering purpose have been conventionally classified into four categories: (I) stereolithography (SLA); (II) selective laser sintering (SLS); (III) three-dimensional printing (3DP); and (IV) fused deposition modelling (FDM). SLA has become a valuable tool for fabrication of biocompatible tissue engineering scaffolds due to its ability to fabricate precise internal architectures and external geometries, which match those of human tissue [22, 25]. This rapid prototyping technique produces complex structures using a layer-by-layer approach that is based on spatially controlled solidification of liquid-based resins by photopolymerization. With respect to accuracy and resolution, SLA is superior to all other RP techniques. While in most fabrication techniques the smallest details are 50–200 μm in size, many commercially available SLA setups can build objects that measure several cubic centimetres at an accuracy of 20 μm [23, 26]. The great versatility in design and precise nature of SLA enables fabrication of scaffolds with complex microstructures. Scaffolds with well-defined pore sizes, porosities, pore distributions, pore interconnectivity, and pore gradients have been fabricated using SLA. Advancements in resin materials as well as incorporation of bioactive materials and fillers have also improved the use of SLA for tissue engineering purpose [13, 27, 28]. In this paper we focus on materials exhibiting a broad range of mechanical properties and biological functionalities that have been developed for SLA, and on their use in the biomedical field.

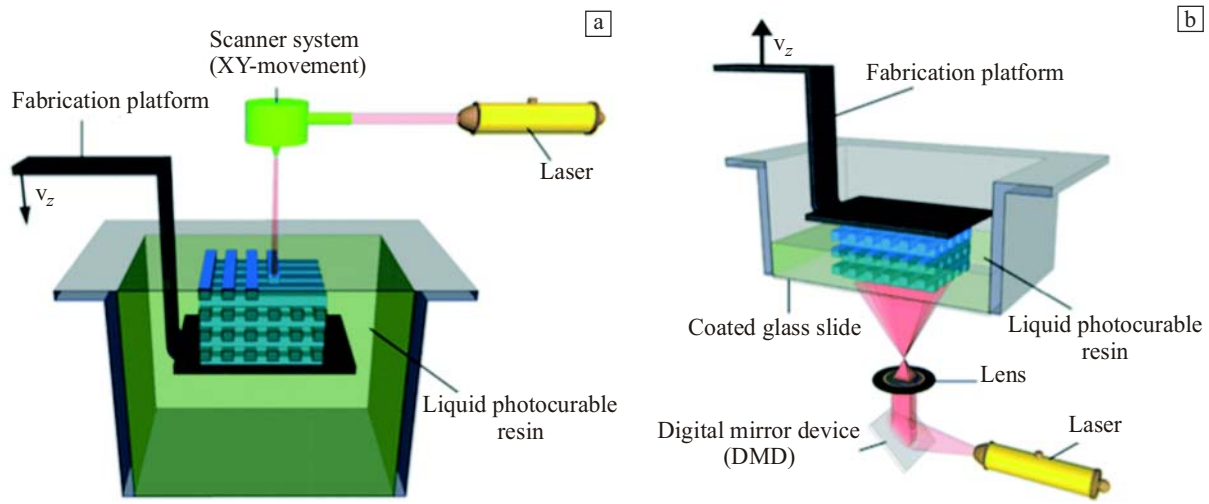

Fig. 2. Different types of SLA setups: (a) bottom-up system with scanning laser, (b) top-down setup with mask-based projection method [29].

2. Stereolithography

Stereolithography is the first commercial RP process developed by 3D Systems Inc. and it is based on layer-by-layer photosensitive resin polymerization using ultraviolet (UV) light. It is an additive fabrication process using a liquid UV-curable photopolymer and a UV laser to build structures one layer at a time (Fig. 2). This relies on a photosensitive monomer resin which polymerizes and solidifies when exposed to UV light [30]. 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 that is solidified to a defined depth, causing it to adhere to a support platform. SLA usually employs two distinct methods of irradiation: laser writing method and the mask-based projection method.

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. The direct or laser writing method is the most commonly used one, involving the use of a focused laser beam to selectively irradiate and solidify the liquid photopolymer [31, 32]. However, this system requires a long process due to the point-by-point laser scanning procedure and post-fabrication procedures. In the mask-based method, an image is transferred to a liquid polymer by irradiation through a patterned mask which contains transparent zones corresponding to the sections of the model to be built [32–34]. This system requires the generation of a great number of masks with precise alignments, which can be done by using Liquid Crystal Display (LCD) panels or Digital micro mirrors devices (DMDTM—Texas Instrument) as a dynamic pattern generator. The mask-based device system has the capability to fabricate an entire layer under one single UV exposure, thereby leading to a high manufacturing speed [35]. In such setups, light is projected on a transparent, non-adhering plate from underneath, and the support or build platform is dipped into the resin from above. 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. A 3D scaffold can be fabricated using this method by projecting a dynamic pattern on a photocurable monomer, resulting in a layer-by-layer fabrication approach. The solid structure in each layer is formed by photopolymerization, the light pattern being controlled by the micro-mirrors in the DMD apparatus. After one layer is solidified, the elevator moves downward and a new layer of liquid resin can be solidified as the next layer [12, 13]. This process is repeated for each layer until the structure is finished. When a part is complete, the building platform is raised from the vat and the excess resin is allowed to drain. At this point the part is not fully cured. After removing any viscous liquid resin that might still be on the surface, the part is placed in an agitated bath of solvent or wiped by hand with solvent to remove any additional uncured resin [7, 11, 12]. After cleaning, the built structure must be post-cured to complete polymerization of the resin and fully harden it. Post-curing takes between one and ten hours depending upon the part size. In this manner SLA can be used to reproducibly prepare three dimensional tissue engineering scaffolds with well-defined pore network architectures. Using SLA it is possible to prepare structures with any shape and also with the gradient of properties, the basic requirement being the ability of the liquid photo-curable resin to form a mechanically stable, solid material upon photo-irra-

diation [22, 23, 36]. The SLA apparatus build parts from layers 25–50 μm thick. It is necessary that the cure depth of these highly loaded suspensions be equal to or greater that 25–50 μm after exposure to radiation in order to fabricate a structure in a reasonable amount of time. Upon exposure to light, a photopolymer obeys the Beer–Lambert law of absorption, and consequently the thickness of the solidified resin layer (cure depth, C_d in μm) is controlled by the light irradiation dose E (in mJ/cm^2). The C_d can be modelled by assuming it to be the depth at which the UV beam is attenuated from the incident intensity E_0 down to the minimum intensity required to achieve photocuring E_c for the particular photoinitiator/monomer system [37]. The theoretical expression for the cured depth C_d is derived from the Beer–Lambert law and can be written as:

$$C_d \propto D_p \ln\left(\frac{E_0}{E_c}\right), \quad (1)$$

where D_p is the depth of penetration of the light [12, 13]. As the applied irradiation dose E_0 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 composite resin is directly related to the extinction coefficient in the Beer–Lambert equation and affects the D_p, a high value of the extinction coefficient of the resin leading to a small D_p [38, 39]. A good resin for SLA is characterized by low values of E_c in order to start the reaction with low energy dose, and by high values of D_p in order to optimize the cured thickness [40]. Precise control of D_p enables minimal overcure and affords accurate control of the building process. For a given resin, the cure depth is determined by the illumination dose (the energy) to which the resin is exposed [41]. This dose can be varied by adjusting the power of the light source or the illumination time. 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. One important feature is the choice of the photoinitiator system because it reacts with the ultraviolet radiation and initiates polymerization. The absorptivity must be tuned to the particular UV wavelength and the concentration maximized for efficient photopolymerization through the depth of the liquid resin [42].

3. Synthetic biodegradable polymers for stereolithography

Synthetic biodegradable polymers have become an invaluable asset in the medical field taking advantages over other synthetic materials because their properties, such as degradation rate and mechanical strength, can be tailored to specific applications. They also induce lower immunological response compared to natural materials. The stereolithographic process transforms a multifunctional pre-polymer into a cross-linked polymer through a chain reaction initiated by reactive species (free radicals or ions) generated by light exposure. Since most monomers or pre-polymers do not produce initiating species upon irradiation, it is necessary to introduce low molecular weight organic molecules (initiators) that will start polymerisation through photophysical and photochemical processes [43]. This reaction is called photopolymerization, and is typically complex, involving many participating species [27, 44]. The stereolithography process makes use of liquid, ultraviolet (UV) curable photopolymers as their primary materials. Frequently, these materials are called simply resins. However, the limited number of resins that are commercially available for processing by stereolithography has often been considered as the main limitation of the technique [32]. The resin is usually composed of the following components: photoinitiator, polymerizable oligomers, a reactive or non-reactive diluent and additives. Most common stereolithographic resins have been free radical systems based on acrylate and methacrylate. These monomers cure rapidly and are easily modified at the ester functionality. However they are relatively volatile, have an unpleasant odour and present potential hazards [45, 46]. Furthermore, there are systems that polymerise through a cationic mechanism like epoxides and vinyl ethers [47, 48] that exhibit low shrinkage and have better mechanical properties than acrylates, but are usually less reactive. The first polymeric systems developed for stereolithography were based on low-molecular weight polyacrylate or epoxy macromers, which form glassy networks upon crosslinking [49, 50]. In the last decades different biopolymers have been modified with photoreactive and crosslinkable groups like acrylates or methacrylates to make the biopolymer functional for processability [13, 51–54].

Gill et al. [51] proposed a reaction route for producing photocurable biodegradable polymers as shown in Fig. 3. The reaction can be divided into three different step: (1) the production of a low-molecular weight pre-polymer by combining a selected monomer and pre-polymer initiator, (2) acrylation of the hydroxyl end-groups to produce a photocurable pre-polymer, and (3) mixing with a photoinitiator and UV irradiation to produce the polymer. By varying the reaction conditions in step 1, different polymers with varying properties can be produced. Among the families of synthetic polymers, polyesters have been attractive for tissue engineering applications because of their ease of degradation by hydrolysis of ester linkage and the potential to tailor the structure to alter degradation rates [55–57]. Among those, poly(α-hydroxy acids) such as poly(glycolic acid) (PGA) [56], poly(lactic acid) (PLA) [57], polycaprolactone (PCL) [58, 59], trimethylene carbonate (TMC) [60] and a range of their copolymers have a long

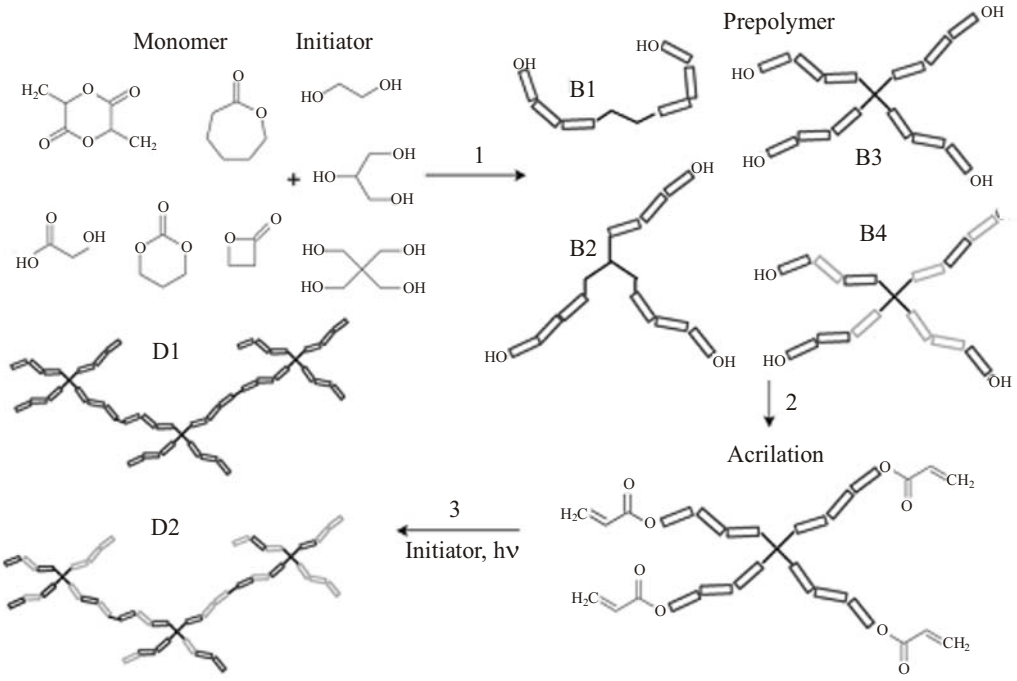

Fig. 3. Polymerization reaction scheme for producing photocurable biodegradable polymers proposed by Gill et al. [51].

history of use as synthetic biodegradable materials and it has been demonstrated that they can be easily modified to be employed in the stereolithography process [12, 13, 23, 27, 53, 61].

3.1. Poly(propylene fumarate)

Poly(propylene fumarate) (PPF) is one of the most widely studied polymers used in stereolithography that exhibits both bio-degradability and photocrosslinking functionality. PPF is an unsaturated linear polyester that degrades into fumaric acid and propylene glycol, both biocompatible degradation products. Once PPF is produced, it can be crosslinked through the fumarate double bond with a vinyl monomer. Sanderson introduced this material in 1988, producing it by trans-esterification of diethyl fumarate and propylene glycol with a para-toluene sulfonic acid catalyst. From that time on, different groups have tried to synthesize PPF by their own methods [62], and they also investigated variation of the terminal groups of PPF in an attempt to improve the mechanical properties of PPF composites without the use of a crosslinking monomer [63]. In the past, PPF was widely studied as a material for bone cement applications due to the similarity of its mechanical properties to those of trabecular bone, whereas in the last decade some studies have focused on using PPF as a material for porous scaffolds with controllable architecture [64]. Cooke et al. demonstrated the feasibility of using the SLA to build 3D scaffolds using a biodegradable, biocompatible resin made of a mixture of diethyl fumarate (DEF), poly-(propylene fumarate) (PPF), and a photoinitiator, bisacylphosphine oxide (BAPO) [64]. A 3D prototype model was successfully manufactured and these scaffolds have application in bone tissue engineering. Similarly, Lan et al. designed and fabricated three-dimensional (3D) porous scaffolds based on a PPF polymer network using micro-SLA (μ-SLA) [65]. Moreover, they examined surface modification of 3D scaffolds by applying accelerated biomimetic apatite and arginine-glycine-aspartic acid (RGD) peptide coating to promote cell adhesion. Lee et al. have optimized the polymer solution composition and laser parameters for the SLA machine. Using PPF as the biomaterial, diethyl fumarate (DEF) as the solvent, and bisacrylphosphrine oxide (BAPO) as the photoinitiator they fabricated three-dimensional scaffolds with hexagonal and cubic pores (prepared from PPF/DEF resin) that can be useful in diverse tissue engineering applications (Fig. 4) [66] .

As reported before, PPF requires a reactive diluent such as diethyl fumarate to obtain an appropriate reaction rate of the resin, and upon photo-polymerisation networks with low glass transition temperatures and low *E*-modulus values under physiological conditions are formed. For tissue engineering of hard tissues such as bone, strong and rigid biodegradable materials are desired. Polylactide is such a material, and it has a long track record of successful application in the clinic and in the preparation of tissue engineering scaffolds [67].

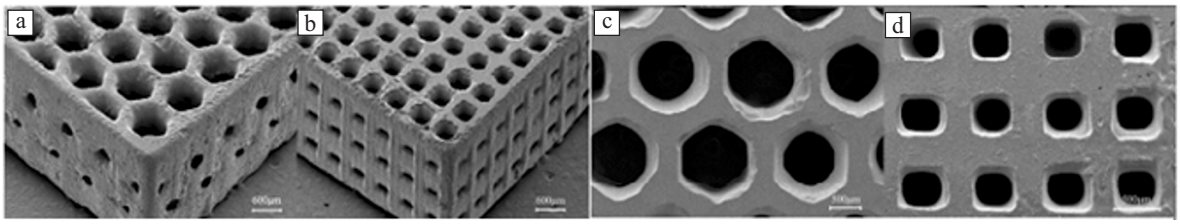

Fig. 4. Scanning electron micrographs of PPF scaffolds with (a, c) hexagon and (b, d) square pore [66].

3.2. Poly (d,l-lactide)

Lactide polymers are well-known polymers that have been applied successfully in medical applications such as resorbable bone fixation devices and in preparation of tissue engineering scaffolds [68, 69]. The amorphous form, poly(d,l-lactide) (PDLLA), has a glass transition temperature of approximately 55 °C, and an elasticity modulus close to 3 GPa; it is one of the few biodegradable polymers with mechanical properties that approach those of bone (the *E*-modulus of bone is 3–30 GPa) [27, 62, 68]. To allow PDLLA network formation by photocrosslinking, double bond-containing lactide oligomers are required. Jansen et al. developed a polymer network by photocrosslinking fumaric acid monoethyl ester (FAME) functionalized, three-armed PDLLA oligomers using N-vinyl-2-pyrrolidone (NVP) as a diluent and co-monomer [49]. The use of NVP together with FAME-functionalized oligomers resulted in copolymerization at high rates and it allows the preparation of tissue engineering scaffolds with mathematically defined architecture and tunable material properties.

Fumaric acid derivatives are attractive compounds since fumaric acid is naturally found in the body, however, compared to the frequently used (meth)acrylate-functionalized oligomers, the reactivity of fumarate-functionalized oligomers is much lower and the use of a reactive solvent is often required for the crosslinking reaction. Melchels et al. prepared, for the first time, porous polylactide constructs by stereolithography without the use of reactive diluents [27]. Star-shaped PDLLA oligomers with 2, 3 and 6 arms were synthesised, end-functionalised with methacryloyl chloride and photocrosslinked in the presence of ethyl lactate as a non-reactive diluent. Materials showed excellent mechanical properties, similar to those of Linear PDLLA and biological characterization showed that mouse pre-osteoblasts readily adhered and proliferated well on these networks. Furthermore mathematically defined porous structures (Fig. 5), with Gyroid architecture, have been realized by stereolithography using the synthesized PDLLA dimethacrylate resin. Methacan et al. also studied the mechanical properties of a photocurable poly(lactic acid) (PLA) resin that was developed for use as a tissue engineering scaffold [70]. Photocrosslinked resins showed a significant increase in mechanical properties compared with commercial bone cement.

3.3. Polycaprolactone

Polycaprolactone (PCL) was one of the earliest polymers synthesized by the Carothers group in the early 1930s as synthetic polymers that could be degraded by microorganisms [71, 72]. Attention was drawn to these biopolymers owing to their numerous advantages like: tailorable degradation kinetics and mechanical properties, ease of shaping and manufacture, and the controlled delivery of drugs contained within their matrix [73]. Due to its properties PCL has been proposed for wide applications such as tissue-engineered skin (plain film), scaffolds for bone regeneration [61, 74, 75] and in long-term drug delivery systems [76, 77]. Functional groups could also be added to render the polymer more hydrophilic, adhesive, or biocompatible which enabled favourable cell responses [78, 79]. Several technologies have been used in order to process PCL for the tissue engineering purpose: Ronca et al. developed a new strategy to repair large segmental bone defect through a combination of particle leaching and filament winding using PCL as a main component of the scaffold [74]. Hutmacher et al. developed a bioresorbable PCL

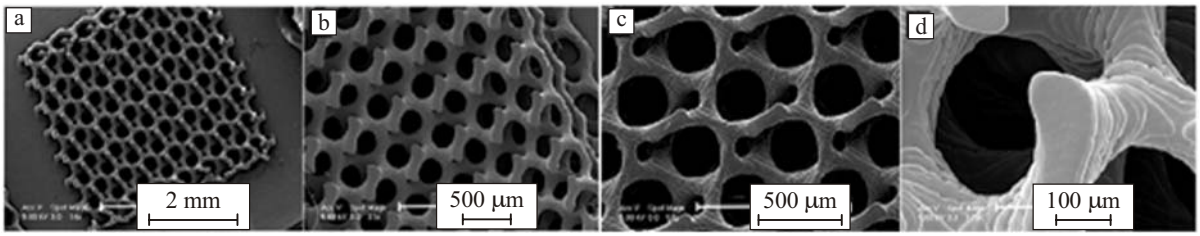

Fig. 5. SEM images of porous PDLLA 3-FAME/NVP Gyroid structures built by stereolithography [49].

as a filament to produce scaffolds by computer-controlled extrusion and deposition method [80]. Williams et al. demonstrates that PCL scaffolds fabricated via selective laser sintering have a great potential for skeletal tissue replacement with mechanical properties in the range of trabecular bone [81]. Photocrosslinkable PCL was developed for the first time by Kweon et al. through reaction of PCL with acryloyl chloride [82]. In this way a PCL macromer that was easily crosslinked via photopolymerization was obtained with enhanced thermal stability and mechanical properties. Grijpma et al. synthesized biodegradable polymer network through functionalization of ε-caprolactone oligomers with fumaric acid monoethyl ester (FAME) [60].

The functionalization method minimizes degradation of the precursor oligomer chains and allows the preparation of networks with a predefined topology. Elooma et al. prepared a 3D porous scaffold by stereolithography using a photocrosslinkable PCL based on methacrylated three-armed oligomers [83]. Photocurable functional groups such as methacrylate have been used most often as an unsaturated group attached on precursor oligomers [84]. However, the diffusion of unpolymerized methacrylates is one of the most important factors causing irritation in tissues [85, 86]. Many studies have found that substances leached out from acrylic resin can cause inflammation, or an allergic reaction [87, 88]. To overcome this problem, Ronca et al. investigated an alternative approach to synthetize a divinyl fumarate PCL that can be used to manufacture tissue engineering scaffolds by stereolithography [59]. Hydroxyethyl vinyl ether was used as ROP initiator and photo-curable vinyl group. Subsequently the vinyl terminated PCL was reacted with fumaryl chloride in order to obtain a tri-functional polymer (Fig. 6).

Biological results indicate good cell adhesion for the tri-functional PCL networks and the material appeared not cytotoxic. Furthermore, the resin was applied to realize mathematically defined complex structures potentially useful for tissue engineering purposes.

3.4. Hydrogels and live cells photocrosslinking

One-step scaffold fabrication with live cell incorporation is a highly desirable technology for tissue engineering and regeneration [89, 90]. In developing such technology, three major factors need to be taken into consideration. First, visible light and a visible light-activated initiator must be used. Second, the cells must remain uniformly suspended in the monomer/initiator solution during structure fabrication. Third, the scaffold itself must be non-cytotoxic and hydrophilic to maintain cell viability after cell encapsulation. Hydrogels are thus suitable as they are capable of trapping water, and their physical properties can mimic those of living tissues. According to Peppas definition, hydrogels are hydrophilic, three-dimensional networks, which are able to imbibe large amounts of water or biological fluids, and thus resemble, to a large extent, a biological tissue [91, 92]. Usually hydrogels are crosslinked polymer networks that possess tissue-like elasticity [93]. In the 1960, Wichterle et al. understood that the extensive application of plastic for alloplastic and prosthetic uses required solution to the problem of compatibility of the material with the living tissue [94]. They synthesized a co-polymer based on glycolmonomethacrylate and glycoldimetachrylate and used it for manufacturing contact lens arteries [94]. One of the most used polymers for hydrogel synthesis is Polyethylene glycol (PEG). PEG is a highly biocompatible material with extensive biological applications. It is suitable for SLA ap-

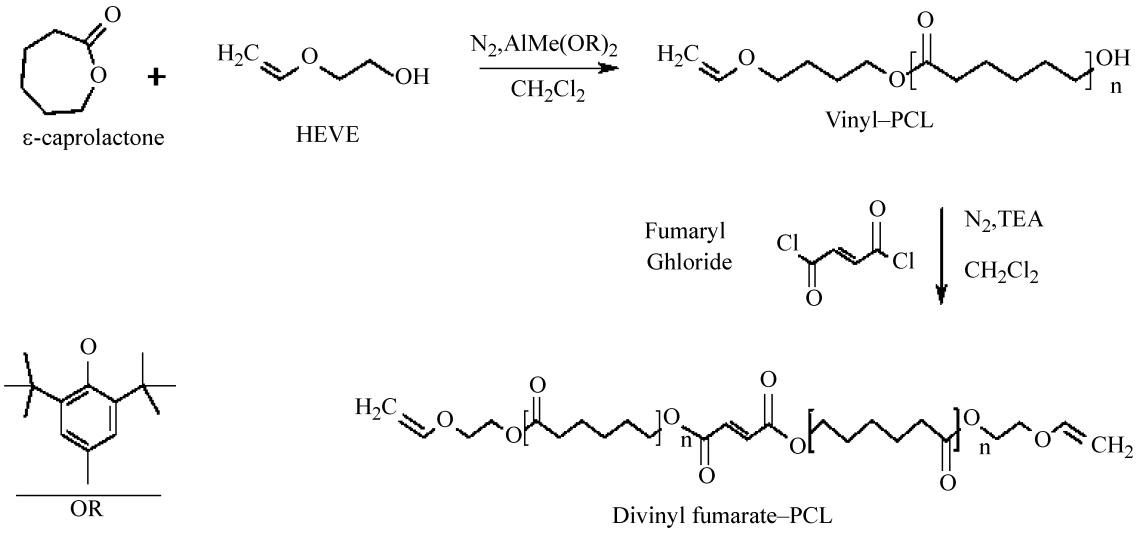

Fig. 6. General reaction scheme of the ε-caprolactone using HEVE as transfer agent and successive reaction with fumaryl chloride proposed by Ronca et al. [59].

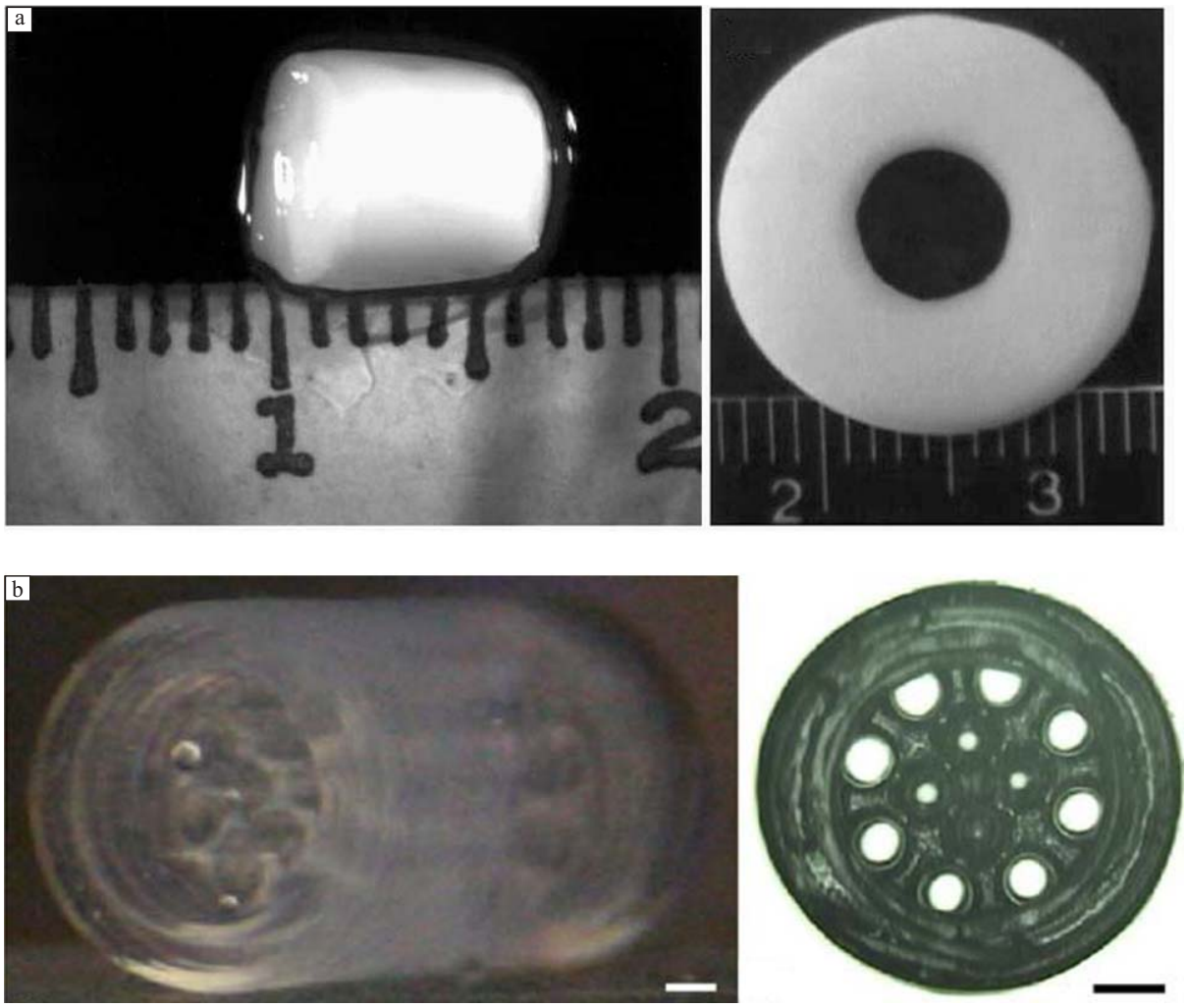

Fig. 7. 3D Peg hydrogel structures: (a) ring designed by AutoCAD and polymerized with a stereolithography machine [97] by Dhariwala et al., (b) complex 3D PEG hydrogel conduit designed by Arcaute et al. [95].

plications because photoreactive and crosslinkable groups like acrylates or methacrylates can be easily attached. These photoreactive groups, in the presence of a photoinitiator and upon exposure to UV light, serve to crosslink the PEG into a hydrogel [95, 96]. Dhariwala et al. used SLA to encapsulate Chinese hamster ovary (CHO) cells in simple shape samples made of poly(ethylene oxide) (PEO) and poly(ethylene glycol)-dimethacrylate (PEGDMA) [97]. Based on the Dhariwale study, Arcaute et al. investigated the use of PEG in SLA for the creation of a complex shape 3D structure for application in tissue engineering (Fig. 7) [95].

In 2013 Lin et al. reported the development of a Visible light projection stereolithography for the fabrication of PEG hydrogels with custom-designed geometry and internal architecture, and the successful incorporation of live human adipose-derived stem cells (hADSCs) within the scaffolds. hADSCs showed high viability in the fabricated scaffolds for up to 7 days post-encapsulation [89]. However, PEG is also void of bioactivity common to many natural hydrogel polymers. As a result, cells are unable to bind directly to PEG hydrogels or modify the microenvironment through enzymatic degradation [98]. Natural hydrogels, such as collagen, gelatin and fibrin have been used as scaffolds because they possess many of critical biological functions like cell adhesion and biodegradation, which are lacking from synthetic polymer [99]. In particular, in the last decade methacrylated modified gelatin has attracted much attention because it is a photopolymerizable hydrogel comprised of modified natural ECM components, making it a potentially attractive material for tissue engineering applications [100]. Hutson et al. demonstrate the ability of Methacrylated gelatin (GelMA) derived from denatured collagen to modify the mechanical and biological properties of PEG hydrogels by altering the concentration of PEG, GelMA, or both [101]. Van Vlierberghe et al. have shown that (methacrylamide-

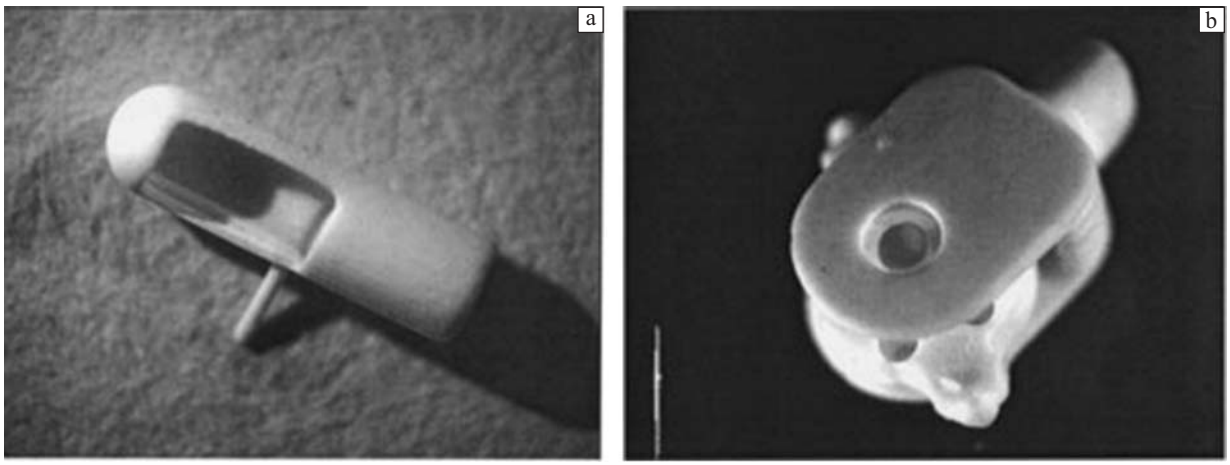

Fig. 8. Two 3D objects made by Provin et al. using a polymer-ceramic photocurable resin. Microcatheter terminal (a), for medical application (3.5-mm long). Microgimbal, for microrobotic application (4-mm long) (b)[110].

modified) gelatin exhibits a high affinity for fibronectin (FN). They demonstrated that the interaction between gelatin and FN is influenced by the gelatin type and structural properties of the scaffold like pore size and pore geometry [102].

3.5. Composites

Nanocomposites have emerged in the last three decades as an efficient strategy to upgrade the structural and functional properties of synthetic polymers [103–105]. The reason for using composite materials is the possibility of designing and tailoring the properties of an implant by just controlling the volume fraction and global arrangement of the reinforcement phase [106]. Therefore, the mechanical properties of nanocomposites are controlled by several microstructural parameters, such as the properties of the matrix, the properties and distribution of the filler, by interfacial bonding, and by the synthesis or processing method [107, 108]. The challenges to extend the use of composite materials to stereolithography is a big research field of the last two decades [13, 23, 109]. To extend its scope to usable composite materials, the SLA method has been adapted for three-dimensional (3D) ceramic fabrication by adding ceramic powders to photosensitive resin. Hinczewski et al. for the first time tried to fabricate a ceramic three dimensional part by stereolithography using ceramic slurry containing alumina powder, UV photocurable monomer diluent and photoinitiator [109]. They first printed 3D structures by stereolithography and subsequently removed the organic components by sintering. To create polymer-ceramic composite objects, ceramic particles (e.g. alumina or hydroxyapatite) must be homogeneously suspended in the resin and photopolymerised in the SLA. The processing of the resin is more difficult, as the viscosity of the resin can significantly increase upon addition of the powder [12]. In 2002, Provin and Monneret extended the use of SLA to the manufacture of ceramic-polymer composite parts. To achieve this, they added dispersed alumina powder (at a weight percentage of 55%) and a visible photoinitiator to a low viscosity diacrylate resin. Thus they were able to produce a small and complex polymer-ceramic object (Fig. 8) [110].

A combination of poly (propylene fumarate) (PPF) and hydroxyapatite nanopowder (nano-HAP) was used by Lee et al. to fabricate nano/microscale composite scaffolds by microstereolithography (MSTL) [111]. The presence of HAP generated nano/microscale topography on the scaffolds surfaces and, in addition, it supported adhesion and proliferation of MC3T3-E1 cells seeded on the scaffolds.

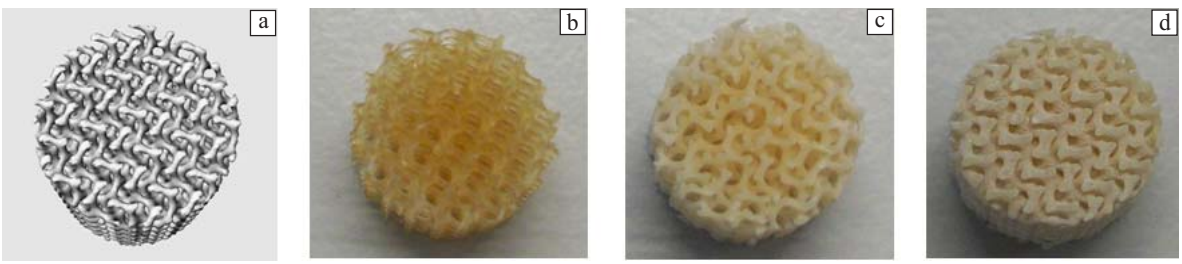

Fig. 9. Double Gyroid nanocomposite scaffolds realized with PDLLA-HA nanocomposite by Ronca et al. using different amount of nanoparticles (DG–CAD (a), DG–X with $X = 0$ (b), 10 (c), 20 wt % (d) of nano-HAP) [23].

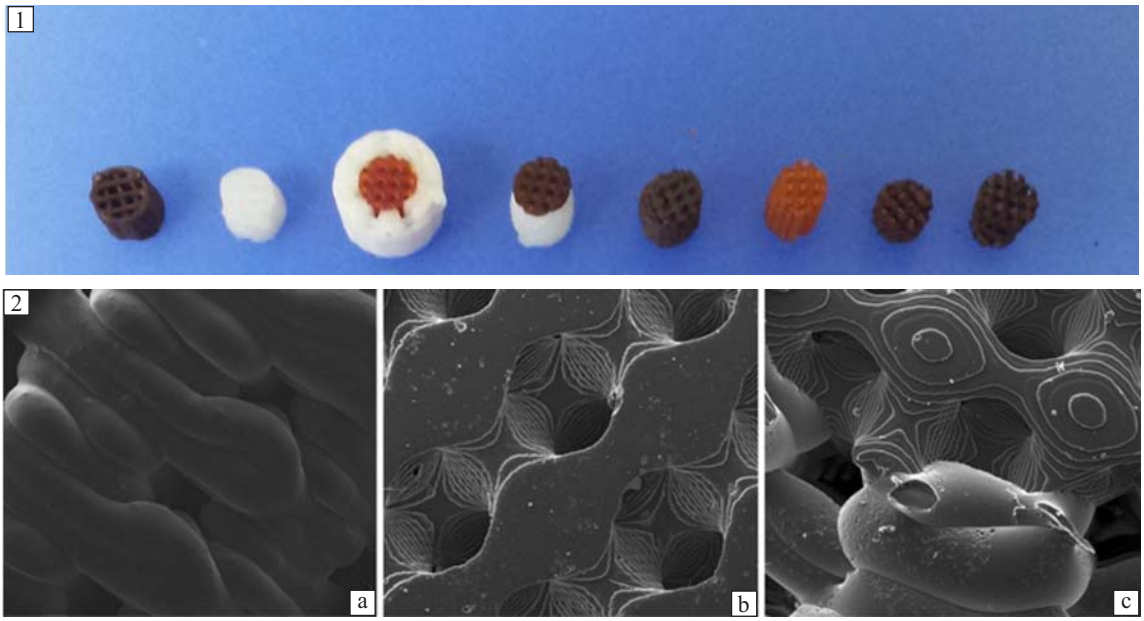

Fig. 10. (1) an image of the scaffolds prototyped through integration of SLA and FDM by De Santis et al. (2) Representative SEM images showing the architecture of the hybrid scaffolds [113].

This approach of using nano-HAP composite photocrosslinkable resin was further developed by Ronca et al. by the realization of mathematically defined porous scaffolds using a PDLLA/nano-HAP resin [13]. Porous scaffolds were computer designed using a Double Gyroid architecture in order to realize a biomorphic nanocomposite scaffold for bone tissue engineering (Fig. 9) [23].

Bian et al. reported an alternative approach that combined laser stereolithography and gel casting technique to realize biphasic osteochondral scaffolds inspired by the bone-cartilage complex microstructure [112]. The scaffolds were composed of beta-tricalcium phosphate (β-TCP) and type-I collagen. Their physical and mechanical properties were tested and the results found are promising for the repair of osteochondral tissue. A similar approach was followed by De Santis et al. in order to realize biphasic scaffolds through the integration of stereolithography and 3D fibre deposition suitable for the regeneration of complex tissues such as osteochondral bone [113]. The region of the scaffold intended for cartilage regeneration was manufactured from polyethylene glycol diacrylate (PEGDA) through stereolithography in order to create a finely structured micro-porous scaffold. The region of the scaffold committed to bone regeneration was manufactured through FDM by the processing of a polymer matrix reinforced with magnetic nanoparticles (Fig. 10).

The possibility to extend the processing of polymer-particle composite to the stereolithographic process has attracted great research interest and finds wide applications in the biomedical field. However, fabricating polymer particle composites with controlled distribution of particles in a host polymer material continues to be a fundamental challenge [114]. To address such a significant challenge, Pan et al. developed a hybrid additive manufacturing process named Projection Electro-Stereolithography (PES), by integrating electrostatic deposition with SLA. Basically, electrostatic deposition is used only for 2D patterning of particle deposition, while the 3D structure is achieved by the stereolithography approach [115]. Compared to existing multi-material 3D printing approach, PES has a unique advantage in local control of particle dispersions, addressing the historical challenge in polymer-particle composite fabrication and opening a new pathway to the design of advanced composite materials through additive manufacturing.

4. Two photon stereolithography

Even if micro-stereolithography (μ-SLA) remains one of the most powerful and versatile of all SFF techniques there are limitations to μ-SL in terms of the spatial resolution of fabricated structures [8]. The minimum thickness of layers is inevitably affected by the viscosity and surface tension of the resin [116] and so it is very difficult to use μ-SL to fabricate ultraprecise microstructures that have a nano-detail or submicrometer scale. In this context, two-photon stereolithography (TPS), established by Kawata and his group [117], appears of high interest since it offers intrinsically sub-100 nm resolution. Additionally, TPS is an attractive fabrication process due to the versatility of materials used including polymers, biopolymers, ceramics, metals, and hybrid materials [118, 119]. Normally in conventional stereolithography techniques polymerization is induced by absorption of a single photon; TPS, instead, is

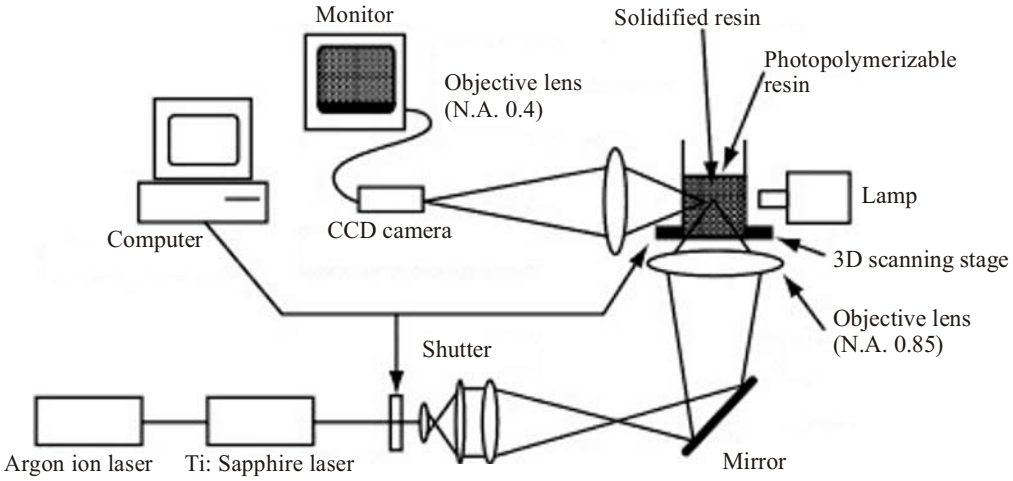

Fig. 11. Schematic of typical two-photon equipment by Gibson et al. [121].

based on a two photon nonlinear absorption (TPA) process [118]. In this system, they used a high-power Ti: Sapphire femtosecond-laser, with wavelength 790 nm; during the process the photoinitiator required two photons to strike and to form a free radical that can initiate polymerization. Owing to these effects, photopolymerization is confined to the focal point of the laser irradiation, in a 3D volume, typically of less than 1 μm^3 [120, 121]. A schematic of a typical research setup for this process is shown in Fig. 11 [121]. Thus, the main advantage of TPS compared to single photon absorption is that excitation is localized within the focal volume of a laser beam. Consequently, it gives access to 3D microfabrication since the polymerization threshold is not reached out of the focal volume [122]. Moreover, another advantage is that parts can be built inside the resin vat, not just at the vat surface, which eliminates the need for recoating and thus greatly improving the production speed.

Nanosized structures formed by this technique have many other promising applications especially in the field of photonic crystals [123], micromechanical parts [124], rapid prototyping of micro/nanofluidics [125], small-scale production of microoptics components [126] and 3D frameworks for cell biology. For example Marksteiner and his group demonstrated that the periodic structures obtained with TPS may have other potential applications in the field of bio-inspired microfluidic devices due to their good wettability and water sustainment [125]. Despite the possibility to fabricate 3D objects with sub-100 nm features in a single step, TPS appears as an extremely slow technique for mass production in industry due to the point by point writing process [118]. This drawback, however, seems to be a fantastic and appealing field of research for the next decades since it is expected that TPS will evolve into a powerful process for the fabrication of 3D nano/microdevices applicable to diverse scientific fields.

5. Conclusions

Stereolithography is a rapid prototyping technique that is particularly versatile with respect to the freedom of design of the structures leading to the fabrication of intricate geometry with exceptional accuracy. In the last two decades optimization of stereolithography processing parameters, novel photopolymerizable resins, and developments in the processing equipment have allowed precise fabrication of structures on the micrometer and submicrometer scale. Overall it has a strong perspective for biomedical applications if used in combination with medical imaging technique like MRI and CT. However, one of the main drawbacks of the stereolithography technique is the limited number of biocompatible resins available for biomedical applications. In the last decades, several studies have developed new resins based on synthetic polymers, hydrogels and also polymer/ceramic nanocomposites that can directly fabricate implantable devices for tissue engineering purpose. Moreover, the introduction in the last few years of the two photon stereolithography allows for microfabrication of micrometer-scale polymeric, ceramic and metallic structures applicable to complex-shape medical implants and optical, electronic and biomedical microdevices.

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