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Review

A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering

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ABSTRACT

The combined potential of hydrogels and rapid prototyping technologies has been an exciting route in developing tissue engineering scaffolds for the past decade. Hydrogels represent to be an interesting starting material for soft, and lately also for hard tissue regeneration. Their application enables the encapsulation of cells and therefore an increase of the seeding efficiency of the fabricated structures. Rapid prototyping techniques on the other hand, have become an elegant tool for the production of scaffolds with the purpose of cell seeding and/or cell encapsulation. By means of rapid prototyping, one can design a fully interconnected 3-dimensional structure with pre-determined dimensions and porosity. Despite this benefit, some of the rapid prototyping techniques are not or less suitable for the generation of hydrogel scaffolds. In this review, we therefore give an overview on the different rapid prototyping techniques suitable for the processing of hydrogel materials. A primary distinction will be made between (i) laser-based, (ii) nozzle-based, and (iii) printer-based systems. Special attention will be addressed to current trends and limitations regarding the respective techniques. Each of these techniques will be further discussed in terms of the different hydrogel materials used so far. One major drawback when working with hydrogels is the lack of mechanical strength. Therefore, maintaining and improving the mechanical integrity of the processed scaffolds has become a key issue regarding 3-dimensional hydrogel structures. This limitation can either be overcome during or after processing the scaffolds, depending on the applied technology and materials.

1. Introduction

To date, organ and tissue transplantation remains one of the most important while complex options in order to restore or enhance life expectancy. The most recent annual report prepared by the Scientific Registry of Transplant Recipients (SRTR) in collaboration with the Organ Procurement and Transplantation Network (OPTN) registered 112,905 patients in the USA awaiting transplantation at the end of 2011, while only 26,246 transplantations were performed [1]. If we keep the steady increase in life expectancy in mind, these numbers emphasize the shortage of organ donors [2]. In addition, diseases, infections and rejection of the tissue by the host often complicate transplantation [3]. To overcome these problems associated with transplantation, the last few decades, tissue engineering (TE) has grown as a new inter- and multi-disciplinary scientific field [4]. This discipline has rapidly emerged and combines the principles of engineering and life sciences. It holds as main objective the recovery, maintenance and improvement of tissue performance [4–6]. The European Commission on Health and Consumer Protection defines TE as the persuasion of the body to heal itself through the delivery, to the appropriate site, independently or in synergy, of cells, biomolecules and supporting structures [7].

Researchers will strive to fulfil those afore mentioned objectives through the utilization of isolated cells [8–11], tissue inducing substances [12–14] and/or scaffolds [3,4,6,15]. Although, conventionally, the application of a supporting scaffold is preferred in circumstances where the defect acquires certain dimensions. Post-processing cell seeding and maturation to tissue has therefore been implemented as a commonly applied TE strategy [15–19]. Expanding the cell population and maturation to tissue is performed in bioreactors, which can be described as devices in which biological and/or biochemical processes are manipulated through close control of environmental and process-bound factors such as pH, temperature, pressure, and nutrient and waste flow [20]. When working with low-water content polymers, post-processing cell seeding is the only available seeding mechanism. However, insufficient cell seeding and/or non-uniform cell distribution have been reported using this methodology [20,21]. There is thus a need for better and more uniform seeding principles. Enhancing the seeding efficiency can, among other, be accomplished by cell encapsulation

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strategies. This method requires a high-water content environment.

Hydrogels based on natural or synthetic polymers have been of great interest regarding cell encapsulation [22–37]. For the past decade, such hydrogels have become especially attractive as matrices for regenerating and repairing a wide variety of tissues and organs [7,12,33–92]. Depending on the hydrophilicity, they can absorb up to thousands of times of their dry weight and form chemically stable or (bio)degradable gels. Depending on the nature of the hydrogel network, 'physical' and 'chemical' gels can be distinguished. Hydrogels are called 'physical' when the network formation is reversible. In contrast to 'chemical' hydrogels, which are established by irreversible, covalent cross-links. Combinations of both physical and chemical networks can also be achieved, e.g. gelatine modified with methacrylamide groups [93].

The characteristic properties of hydrogels make them especially appealing for repairing and regenerating soft tissue [32,37–39,85–92,94–97]. One of the main disadvantages of processing hydrogels is the difficulty to shape them in predesigned geometries. This article will provide a detailed overview of the different rapid prototyping techniques that are compatible with hydrogel manufacturing and allow to accurately shape external and internal geometries. Since we did not find an article that summarizes the potential advantages and disadvantages regarding the processing of hydrogels with RP techniques, it is the purpose to highlight the advantages, but more importantly also the current limitations of the distinctive techniques, together with the respective hydrogels used so far.

In the first part, an introduction to scaffolding and basic concepts of scaffold-based and scaffold-free TE will be given. The next part handles hydrogel-friendly RP techniques used in scaffold-based TE. Finally, the implementation of RP technology in scaffold-free TE will be explained.

2. ECM mimetics: Current concepts

2.1. Scaffold-based vs. scaffold-free TE

From a cell biology perspective, 2D cell culture models only provide physiologically compromised cells induced by an unnatural environment [98], and the lack of a 3D structure will cause cells to form a random 2D mono-layer [17,19]. *In vivo*, cells are subjected to growth in three dimensions and complex cell–cell interactions. This observation encouraged a paradigm shift from conventional 2D cell culture models towards 3D microenvironments [99]. To obtain a more realistic understanding of cell–cell and cell–biomaterial interactions, Kirkpatrick et al. [100] proposed the use of co-culture models *in vitro*. Independent of the applied strategy, the ultimate goal of TE remains the same. Nevertheless, regarding the aspect of 3-dimensional cell migration, proliferation and differentiation behavior and requisites, one can distinguish two major premises. Currently, both of them are being heavily explored. The first one is based on the presumption that cells require a 3D biomaterial scaffold that closely mimics the corresponding extracellular matrix (ECM) [99,101]. In this approach, the biomaterial construct acts as a necessary cell guide and supporting template. The second one finds its roots in the hypothesis that cells have a considerable potency to self-organize through cell–cell interactions and is referred to as 'scaffold-free TE' [102]. While the former theory maximizes the role of a supporting structure as a cell guide and minimizes the potency to self-assembly, the latter reverses the importance of both contributions.

2.2. Scaffolds

Ideally, scaffolds can be seen as ECM biomimetic structures with three main objectives [17,18]: (i) defining a space that moulds the regenerating tissue; (ii) temporary substitution of tissue functions, and; (iii) guide for tissue ingrowth. It is clear that scaffold design should meet the needs of some basic requirements to be able to meet those objectives, including [3,15,17–19]: high porosity (preferably 100% interconnectivity for optimal nutrient/waste flow and tissue ingrowth); relevant geometry and pore dimensions (5–10 times the cell diameter); biodegradable with adjusted degradation time; maintaining the mechanical integrity during a prefixed time frame; it should have suitable cell–biomaterial interactions, and; be easy to manufacture. Adjusting the mechanical and degradation properties to the desired tissue is essential. Either enzymatic or non-enzymatic hydrolytic processes control the degradation profile. Specifically, TE requires biomaterials that provoke cell interactions (~bioactivity) [103] and as little as possible adverse body reactions (~biocompatibility) [104]. Control over the material bioactivity can be achieved by incorporating growth factors [105], enzymatic recognition sites [106], adhesion factors [94,107], or material modifications [106]. Material modification is a general term indicating either bulk modification [103,108] or surface modification [103,109,110]. Modifying the bulk properties is closely related to material biocompatibility, the physical and chemical properties covering the life-span of the implant [111], while varying the surface chemistry reflects on the initial cell/tissue–material interactions [111,112]. Fig. 1 illustrates schematically the complex multi-disciplinary interactions inherent towards scaffold fabrication. In the sub-science of scaffolding, both conventional and rapid prototyping (RP) techniques have been explored. Conventional scaffold fabrication setups include techniques such as particulate leaching [85,113–115], gas foaming [114–117], fibre networking [118,119], phase separation [120,121], melt moulding [122,123], emulsion freeze drying [124,125], solution casting [126,127], freeze drying [81,87,128] and combinations of those. Conventional/classical approaches are defined as processes that create scaffolds with a continuous, uninterrupted pore network. Nonetheless, they completely lack long-range micro-architectural channels [19]. Other reported disadvantages involve low and inhomogeneous mechanical strength, limited porosity and insufficient interconnectivity, inability to spatially design the pore

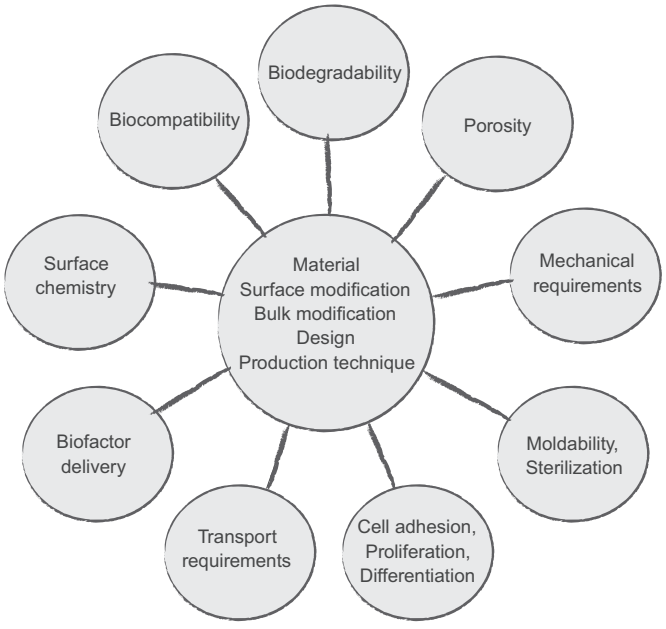


Fig. 1. Schematic illustration integrating the complex multi-disciplinary needs which determine the constraints for the ideal scaffold fabrication design.

distribution (internal channels) and pore dimensions, and difficulty in manufacturing patient specific implants (control over external geometry is limited) [19,129]. Furthermore, the use of organic solvents during processing is seen as a second major drawback in addition to the above mentioned architectural drawbacks. The presence of organic solvent residues can pose significant constrains related to toxicity risks and carcinogenetic effects [19]. Despite some adaptations, over the years the scaffold design remains process-dependent by means of classical approaches. A more design-dependent method would be attractive, and this can be attained by RP techniques.

2.3. Controlling the external and internal geometry

Solid freeform fabrication (SFF) is the general term covering all techniques that produce objects through sequential delivery of energy and/or material. When rapid fabrication of a prototype, a finished object or a tool is pursued, they are respectively called rapid prototyping (RP), rapid manufacturing (RM) and rapid tooling (RT) [130]. By means of RP, an additive computer-controlled layer-by-layer process generates a scaffold. 3D computer models shape the external design, and such models can either be designed by CAD software or by modelling imaging data (CT, MRI). On the other hand, the internal architecture is determined by the processing of the CAD data into an STT file and subsequent slicing of the STT data (generation of the machine parameters). This directly indicates one of the greatest assets of RP: direct fabrication of scaffolds with a complex, patient specific external geometry in combination with a precise control over the internal architecture (limited by the resolution of the system) [17,19,65]. Other advantages comprise: high degree of interconnectivity, possibility to use heterogeneous materials, high speed due to a high degree of automization and the limited number of process steps, and a superior cost-efficiency [3,65]. Both direct and indirect RP methods exist. In the former case, the scaffold is directly processed from a biomaterial, in the latter case the scaffold is processed out of an RP mould. Worldwide, more than 30 different RP techniques are being applied in the most diverse industries, and around 20 of them found applications in the biomedical field [130]. Several authors have reviewed this particular subject [3,130,131]. Although the wide diversity of RP technologies, only some of them seem to be compatible for the processing of hydrogels. The next chapter describes the different RP scaffolding techniques compatible with hydrogels.

3. Rapid prototyping hydrogels: Powerful aid in making scaffold-based tissue engineering work

A primary classification of the SFF techniques supporting biomedical applications can be made hinged on the working principle: (i) laser-based; (ii) nozzle-based, and; (iii) printer-based systems. Laser-based systems benefit from the photo-polymerization pathway as a basis to fabricate cross-linked polymeric TE scaffolds. The well-known processing of (pre)polymers by dint of extrusion/dispension supports the second category of RP systems. The last subclass works with powder beds and deposition of a binder that fuses the particles, or directly depositing material using inkjet technology. An important characteristic feature of every technique will be its resolution. Every technique is subdued to a lower technical limit size of the smallest details producible. This so-called lower limit shows a clear relationship with the feasible scale of the object: the higher the resolution of the smallest details, the smaller will be its maximum object size [132]. However, since not all RP techniques are applicable for the processing of hydrogel materials, some more than others, the amount of RP technologies is further diluted. Fig. 2 classifies the different RP techniques with biomedical applications. The fabrication of hydrogel scaffolds requires mild processing conditions. Some of the techniques mentioned in Fig. 2 are not able to meet those constraints due to the rather harsh processing conditions. Exploring on all of those techniques is not the purpose of this review in which the hydrogel compatible systems only will be explained in detail.

3.1. Laser-based systems

3.1.1. Working principles and recent trends of laser-based systems

With the exception of selective laser sintering (SLS), all of the laser-based systems are suitable for hydrogel processing. Unlike the nozzle- and printer-based systems that sequentially deposit material, this subclass sequentially deposits light energy in specific

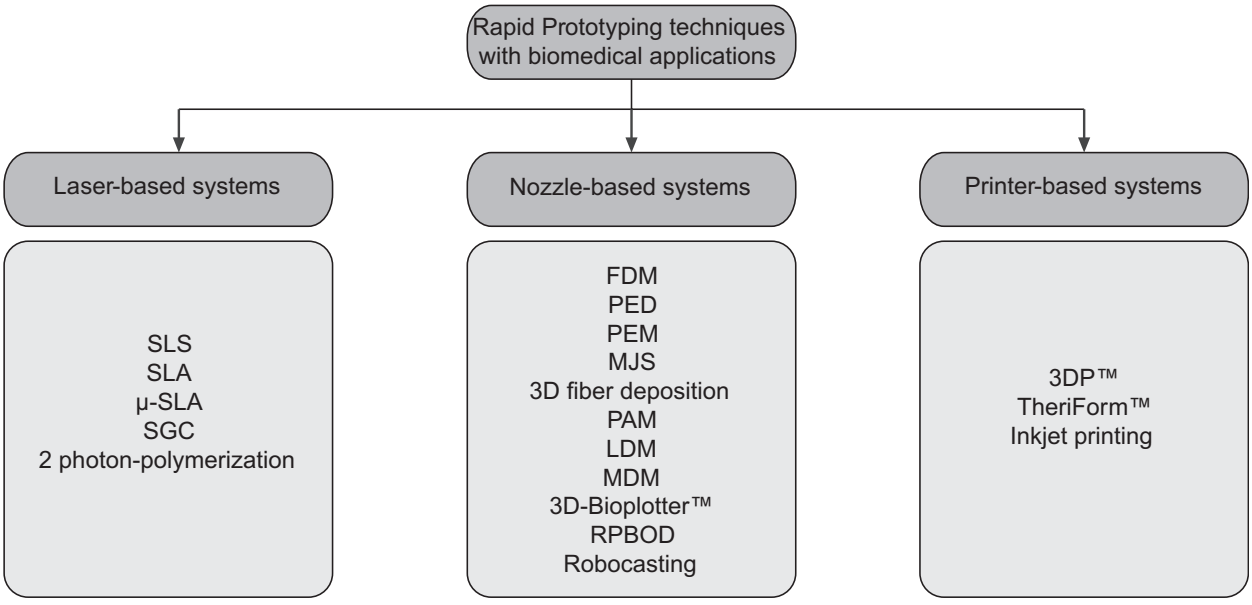


Fig. 2. Classification of RP techniques with biomedical applications into laser-, nozzle-, and printer-based systems.

predefined patterns. This directly implies that only photocross-linkable prepolymers can be employed to finally obtain a cross-linked hydrogel network.

SLA: Stereolithography (SLA) is considered to be the first commercially available SFF technique, developed by 3D systems in 1986 [132]. An SLA apparatus (Fig. 3) consists of: a reservoir to be filled with a liquid photocurable resin, a laser source (commonly UV light), a system that controls the XY-movement of the light beam, and a fabrication platform that permits movement in the vertical plane. Scanning the surface of the photosensitive material produces 2D patterns of polymerized material through single photon absorption at the surface of the liquid material. The build-up of a 3D construct is made possible using a layer-by-layer approach, whereby the fabrication platform moves stepwise in the *Z*-direction after a 2D layer is finished. The step height of the fabrication platform is typically smaller than the curing depth, ensuring good adherence of subsequent layers. Post-treatment steps involving washing-off excess resin, and further curing with UV light are in most cases necessary. Arcaute et al. [133] demonstrated the possibility to alter the resolution of the cure depth by varying the laser energy, the concentration of poly(ethylene glycol) dimethyacrylate (PEG-DMA) as photocross-linkable material, and the type and concentration of the photo-initiator. In addition, adjusting the scanning speed influences the cure depth.

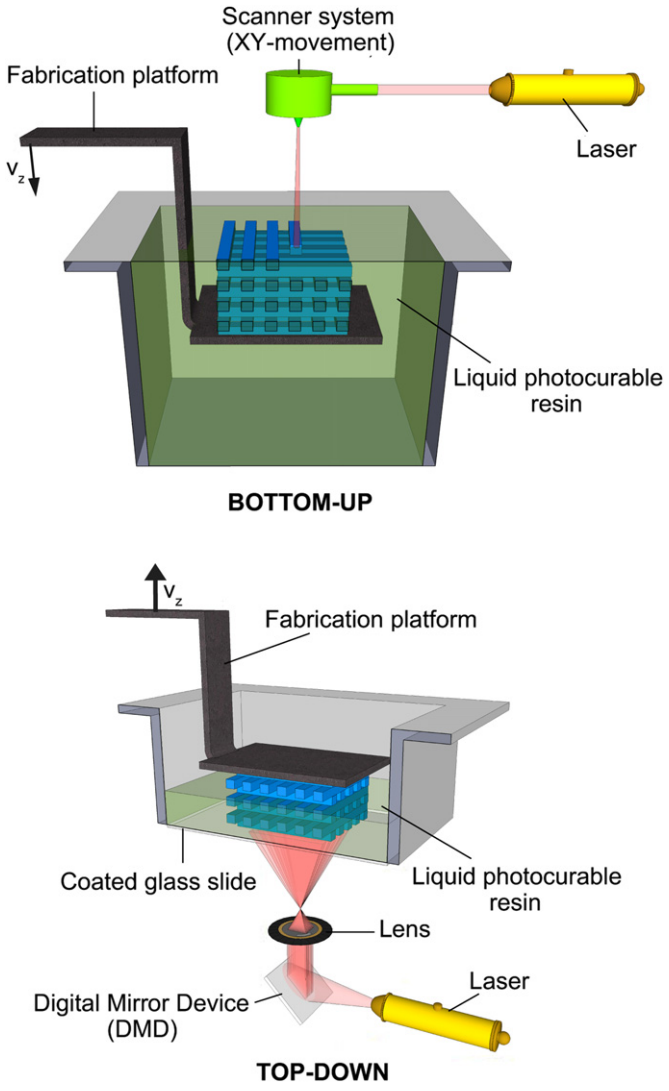


Fig. 3. Scheme of bottom-up and top-down stereolithography setups. The bottom-up setup shown is an example of a system whereby the laser scans the surface for the curing of the photosensitive material. In the example of the top-down setup, dynamic light projection technology is used to cure a complete 2D layer at once.

μ-SLA: The working mechanism of micro-stereolithography (μ-SLA) can be considered the same as that of a normal SLA. The difference between both involves the resolution of the system. μ-SLA systems are typically able to build very accurately (a few microns) objects of several cubic centimeters [134–137].

Fig. 3 (top) shows a scheme of a so-called bottom-up setup, in which an object is built from a fabrication support just below the resin surface. Subsequent layers are being cured on top of the previous layers by irradiation from above. Although, to date this is the most applied setup [133,135–140], a top-down approach (Fig. 3 bottom) is gaining interest [71,132,134]. Top-down setups have a non-adhering, transparent plate acting as the bottom of the liquid reservoir. Polymerization of the photosensitive material occurs through irradiation from underneath, and the fabrication platform moves in the opposite direction as in the bottom-up approach. In this way, every newly formed layer is located beneath the previous one. Separating every newly formed layer from the bottom plate will subject the structure to larger mechanical forces but on the other hand the vat content can be minimized, the irradiated surface will not be exposed to the atmosphere (cross-linking efficiency limitation due to oxygen will be minimized), recoating the structure with a new resin layer is not required, and the illuminated area is always smooth [132]. Another recent and more fundamental trend in the field of SLA is the emerging use of digital light projection (DLP) technology [134,140–142]. The working principle is illustrated in Fig. 3 (bottom) in the top-down scheme, but is also applicable for bottom-up setups. Projection technology enables the curing of a complete layer of resin in one go, which obviously reflects on the building time. A Digital Micro-mirror Device™ (DMD) consists of an array of mirrors, which can independently be tilted in an on/off state. In this way the DMD serves as a dynamic mask that projects a 2D pattern (often designed in PowerPoint slides) on the surface. Instead of DMD, LCD displays have also been employed as a dynamic mask projector [143,144], however, DMD offers better performance in terms of optical fill factor and light transmission [140].

SGC: A projection technology somewhat similar to DMD technology is solid ground curing (SGC) developed by Cubital Inc. [145,146]. Coating the fabrication platform by spraying a photosensitive resin is the first step in the SGC workflow. Meanwhile, the machine prints a photomask of the layer to be built on a glass plate above the fabrication platform. The printing process resembles the one applied in commercial laser printers. Solidification of the sprayed layer occurs when the mask is exposed to UV light, only permitting irradiation of the transparent regions. After the layer is completed, excess liquid resin is removed by vacuum and replaced by a wax to support the next layer. Before repeating the cycle, the layer is milled flat to an accurate, reliable finish for the next layer.

2PP: Two photon-polymerization (2PP) is an emerging state-of-the-art laser-based technique. In this process, light is used to trigger a chemical reaction leading to polymerization of a photosensitive material. Unlike other light curing systems (single photon-polymerization), 2PP initiates the polymerization through irradiation with near-infrared femtosecond laser pulses of 800 nm (Fig. 4). In the focal point, a suitable photoinitiator absorbs two photons, with a wavelength of 800 nm, simultaneously, causing them to act as one photon of 400 nm, and thus starting the polymerization reaction [134]. The nonlinear excitation nature triggers polymerization only

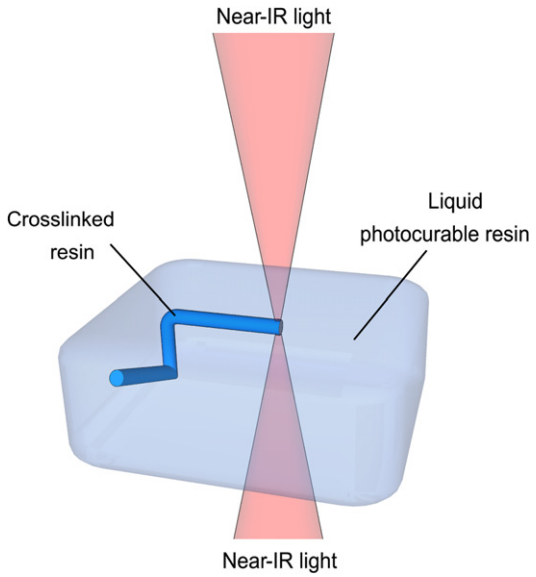


Fig. 4. Working principle of two-photon photopolymerization. In the focal point of the near-IR laser beams, the photosensitive polymer is cross-linked. A 'true' 3D object is obtained.

in the focal point, while other regions remain unaffected. This approach has potential solidification resolutions below the diffraction limit of the applied light. Moving the laser focus enables the fabrication of a direct 'true' 3D object into the volume of the photosensitive material. Creating reproducible micron-sized objects with feature sizes of less than 100 nm is attainable, thus being superior to all other SFF techniques regarding accuracy and resolution [147].

3.1.2. Current limitations and hydrogel feasibility for laser-based systems

SLA has already been frequently applied to develop porous hydrogel-based scaffolds (Table 1). Yu et al. [148] have described the patterning of 2-hydroxyethyl methacrylate (HEMA) followed by drying and subsequent rehydration to enable cell adhesion. Initially, the procedure was applied to create single-layer structures, however, at present, multiple layers can be superimposed to generate porous 3D scaffolds. Liu et al. [31] selected poly(ethylene glycol) diacrylate (PEG-DA) hydrogels to construct 3D scaffolds layer-by-layer using emulsion masks. In that study, three hydrogel layers were fused at a resolution of several hundreds of microns. Interestingly, the pores were interconnected enabling cell survival through convective flow of culture medium. However, this procedure is time consuming, requires a great number of prefabricated masks depending on the required shape and is not completely automated. Therefore, one can discuss whether this can be considered as a genuine rapid prototyping technique. Lu et al. [140,149] have adopted the above-mentioned procedure to develop scaffolds possessing complex internal architectures and spatial patterns within a *Z*-range of several hundreds of microns. Another research group even produced PEG-based scaffolds in the millimeter scale [133,150–152]. Several other research groups also selected PEG-DA as starting material [32,153]. Yasar et al. [32] successfully plotted 100 μm-sized complex scaffold architectures. The swelling effects of the PEG-DA, however, prevented the fabrication of highly reproducible samples below 100 μm. Higher resolutions could be obtained in the presence of UV absorbers since they prevent the internal reflection of the UV light within the polymer solution. More recently, the feasibility of plotting cell-encapsulated hydrogels has been evaluated. Several research groups have already reported on the cell encapsulation in photocross-linkable poly(ethylene glycol) (PEG) microgels [31,154].

Table 1
Hydrogel materials explored in laser-based systems.

Laser-based systems	Hydrogel materials	Cell encapsulation	Reference
	Gelatin-methacrylate	×	[71,134, 159]
	Gelatin-methacrylamide	×	
	Hyaluronic acid-methacrylate	Murine fibroblasts (NIH-3T3) Murine embryonic stem (ES) cells	[155,157]
	Cystein-modified agarose	×	[156]
	HEMA	×	[148]
SLA	PEG-D(M)A	Human hepatoma cells (HepG2) Hepatocytes NIH 3T3 Human dermal fibroblasts (HDFs) Chinese hamster ovary (CHO) cells Murine OP-9 marrow stromal cells Mammalian cells	[31,32,133, 138,140, 154]
	PEG-D(M)A	×	[142, 150–153]
	Alginate + Acrylated TMC/TMP	Chondrocytes	[135–137]
μ-SLA	Gelatin-methacrylamide	×	[134]
	PEG-DA	×	[158,159]
	Gelatin-methacrylamide	×	[134, 163–165]
	Fibronectin	×	[165]
2PP	Bovine serum albumin (BSA)		
	PEG-DA	×	[160,161]

In addition to synthetic polymers, photocross-linkable biopolymers including hyaluronic acid (HA) [155] and gelatin [71] derivatives have already been printed with or without cells using SLA.

In order to enhance the cell-interactive properties of a material, different surface functionalization strategies can be elaborated [32]. Luo et al. [156] grafted RGD-containing peptide sequences on the surface of scaffolds composed of cystein-modified agarose. Han et al. [142] applied a fibronectin coating on the surface of a PEG-DA scaffold to improve the attachment of murine marrow-derived progenitor cells. However, important limitations of laser-based systems include both the need for photocross-linkable materials as well as the effect of the applied UV light on the encapsulated cells [157].

Since shrinkage occurs after post-processing of scaffolds developed using SLA, a major drawback is its limited resolution [139]. In addition, due to scattering phenomena of the applied laser beam, a significant deformation occurs when relatively small objects are developed. The produced hydrogel is often weak upon removal and post-curing is often essential.

Therefore, μ-SLA was introduced to counter the limitations of SLA from a resolution point-of-view. For example, Lee et al. [135–137] developed a hybrid scaffold consisting of an acrylated TMC/TMP (trimethylane carbonate/trimethylolpropane) framework and an alginate hydrogel for chondrocyte encapsulation. The encapsulated cells retained their phenotypic expression within the structure and the scaffold remained mechanically stable up to 4

weeks after implantation in mice. Barry et al. [158] have combined direct ink writing (DIW) with *in situ* photopolymerization to create hydrogel scaffolds possessing μm-sized features. Using this approach, another research group even realized submicron range structures based on PEG-DA [159].

In addition to SLA techniques, SGC also shows potential to be applied in the development of porous hydrogel-based scaffolds for tissue engineering, as already indicated before [139]. However, up to now, no literature data regarding this application and hydrogel processing can be found.

In order to achieve 3D subcellular resolution during scaffold development, 2PP can offer a suitable alternative for SLA. Since this technique was only properly introduced recently, only few reports can be found in literature regarding the application of 2PP to produce porous hydrogels. For example, Schade et al. [160] developed hydrogel-like scaffolds possessing well-defined 3D structures using a methacrylated polyurethane and PEG-DA as starting materials. Ovsianikov et al. [161] also selected PEG-DA as starting material for 2PP scaffolds. More recently, they evaluated the feasibility to produce porous scaffolds using methacrylamide-modified gelatin developed in our research group [162]. The results were very recently published and demonstrated the techniques potential in the processing of biopolymers [163,164].

Table 5 summarizes more technical details on the implementation of hydrogels in the different RP technologies discussed.

3.2. Nozzle-based systems

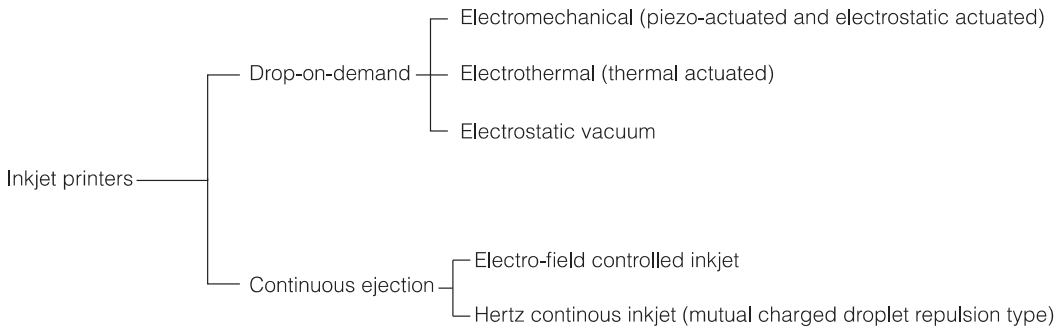
3.2.1. Working principles and recent trends of nozzle-based systems

The class of nozzle-based systems is characterized by a wide diversification (Fig. 2). Fused deposition modelling (FDM), 3D fibre-deposition, precision extrusion deposition (PED), precise extrusion manufacturing (PEM), and multiphase jet solidification (MJS) are techniques based on a melting process. Generally, the melt process involves elevated temperatures, which are undesirable from the perspective of scaffold bioactivity [18]. Researchers have therefore tried to bring forth several other techniques that overcome this limitation by applying a dissolution process, which is attractive for

Table 2
Hydrogel materials explored in nozzle-based systems.

Nozzle-based systems	Hydrogel material	Cell encapsulation	Reference
	Gelatin/Hyaluronan	×	[203]
	Gelatin/Alginate/Fibrinogen	Adipose-derived stromal	[196,204–206]
	Gelatin/Fibrinogen	cells (ADSC)	
	Gelatin/Alginate	Pancreatic Islets	
	Gelatin/Chitosan	Hepatocytes	
	Collagen-chitosan-hydroxyapatite	×	[207]
	Gelatin	Hepatocytes	[208]
	Gelatin-ethanolamide methacrylate,	HepG2	[197]
	Hyaluronan-methacrylate	Human intestinal epithelial	
		cells (Int-407)	
		NIH 3T3	
3D BIOPLOTTER	PCL hybrid with alginate	Chondrocyte cell line (C20A4)	[209]
3D BIOASSEMBLY TOOL	Collagen	×	[193,194]
	PCL/PLGA hybrid with acetocollagen,	Hepatocytes	[210]
	Gelatin or hyaluronic acid	MC3T3-E1 Preosteoblast	
3D CELL ASSEMBLER	Gelatin, Agar	×	[211]
	PEG-DA/Alginate	×	[202]
	PEG-DA	×	[212]
	Pluronic F127 AlaL	Multipotent stromal	[213]
		cells (MSCs)	
	Pluronic F127	Bone marrow stromal	[44,193]
		cells (BMSCs)	
		Human fibroblasts	
		Bovine aortic endothelial	
		cells (BAECs)	
	Alginate	BMSCs	[44,201,214]
	Matrigel	Hepatocytes	
	Methylcellulose	Endothelial cells	
	PEO-PPO-PEO block copolymer		
	Agarose		
	Silicone sealant Silicon SE®	×	[179]
	Polyurethanes	×	[215]
RPBOD	Chitosan	×	[181]
DIW	Acrylamide/Glycerol/Water	×	[158]
	Polyacrylate latex particles in	×	[216]
	Pluronic® F127		
	PEI-coated silica microspheres	×	[185]
	Polyelectrolyte complexes of	×	[186]
	polyanions (PAA) and polycations (PEI, PAH)		
	Titanium diisopropoxide bisacetylacetonate	×	[187]
	(TIA) and PVP		
(M) – LDM	Gelatin/Alginate/Fibrinogen	Hepatocytes	[191]
	Gelatin/Alginate/Chitosan	Primary rat ADSC	
	Gelatin, Sodium alginate	Schwann cells	[192]
		Primary neuron cells	
	Gelatin/Chitosan and type I collagen	×	[167]
PAM	Polyurethane elastomer (Polytek 74–20)	×	[217]
ROBOCASTING	Pluronic® F127	×	[183]
EXTRUDING/ASPIRATION PATTERNING SYSTEM	Mebiol gel (N-isopropylacrylamide and polyoxyethyleneoxide)	Sf-9 insect cells	[190]

Table 3
Classification of inkjet printers.



the processing of hydrogels. Four major nozzle designs have been described in literature: pressure-actuated, solenoid-actuated, piezoelectric, and volume-actuated nozzles [166,167]. These nozzle types can be found in the following systems.

PAM: A technique that resembles FDM without the need for heat is the pressure-assisted microsyringe (PAM) technique, developed by Vozzi et al. [168]. The setup consists of a 5–20 μm pneumatic-driven glass capillary syringe that can move in the vertical plane and deposits material on a substrate. The substrate proceeds in the planar field relative to the syringe. Transforming jpeg or bitmap images into a sequential list of linear coordinates easily allows depositing practically any type of structure in subsequent layers [169]. Material viscosity, deposition speed, tip diameter and the applied pressure correlate with the final deposited strand dimensions. The PAM system has been described in several publications [170,171]. Recently, the fabrication of hydrogel scaffolds was successful with the PAM method [172,173].

LDM: Proposed by Xiong et al. [174] in 2002, low-temperature deposition modelling (LDM) found his way as an RP system with biomedical applications. The key feature of this technique is a non-heating liquefying processing of materials [174]. Using temperatures below 0 °C, the material solution is solidified when deposited on the fabrication platform [175]. The material gets extruded out of a nozzle capable of moving in the XY-plane onto a build platform movable in the *Z*-direction. Incorporating multiple nozzles with different designs into the LDM technique gave existence to multi-nozzle (low-temperature) deposition modelling (MDM, M-LDM) [166,167,176,177]. A multi-nozzle low-temperature deposition and manufacture (M-LDM) system is proposed to fabricate scaffolds with heterogeneous materials and gradient hierarchical porous structures by the incorporation of more jetting nozzles into the system [178]. Biomolecules can be applied in the LDM process to fabricate a bioactive scaffold directly.

Table 4
Hydrogel materials explored in printer-based systems.

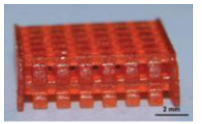
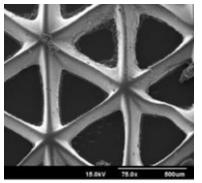
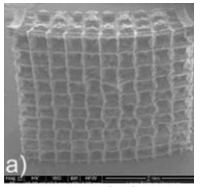
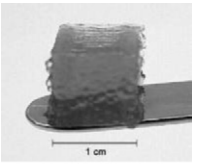
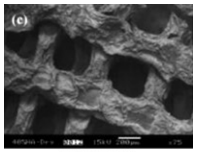
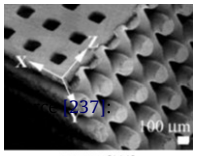
Printer-based systems	Hydrogel material	Cell encapsulation	Reference
3DP™	Starch/Cellulose/Dextrose	×	[221,222]
	Starch/Cellulose fiber/ Sucrose/Maltodextrin	×	[235]
	Corn starch/Gelatin/Dextran	×	[222]
	Starch/Polyurethanes/PEG	×	[221]
	PEO/PCL	×	[226]
	PLLGA/Pluronic® F127	×	[229]
	HA/Cellulose/Starch	×	[234]
	PEG/Collagen/PDL	×	[227]
Inkjet printing	PNIPAM, Collagen	Bovine aortal endothelial cells	[236]
	Alginate/Gelatin	Mouse endothelial cells (ATCC CRL-2581)	[224]
	Fibrin	Rat primary hippocampal and cortical cells	[228,230]
		Human microvasculature endothelial cells (HMVEC)	

3D-Bioplotter™: This 3D dispensing process, displayed in Fig. 5, has been introduced by Landers and Mülhaupt in 2000 at the Freiburg Research Centre [179]. The technique was specifically developed to produce scaffolds for soft tissue engineering purposes, and simplifying hydrogel manufacturing. The three-dimensional construction of objects occurs in a laminar fashion by the computer-controlled deposition of material on a surface. The dispensing head moves in three dimensions, while the fabrication platform is stationary. It is possible to perform either a continuous dispensing of microstrands or a discontinuous dispensing of microdots. Liquid flow is generated by applying filtered air pressure (pneumatic nozzle), or using a stepper-motor (volume-driven injection (VDI) nozzle). The ability to plot a viscous material into a liquid (aqueous) medium with a matching density is the key feature of this process. Low viscous materials in particular benefit from this buoyancy compensation principle. Since heating is not required, the system can process thermally sensitive bio-components, and even cells. Curing reactions can be performed by plotting in a co-reactive medium or by two-component dispensing using mixing nozzles. The strand thickness can be modulated by varying material viscosity, deposition speed (speed in the planar field), tip diameter, or the applied pressure. Constructs build by this plotting technique mostly have smooth strand surfaces, which are not desired for appropriate cell attachment. Therefore, further surface treatment is required to render the surface favourable for cell adhesion. Recently Kim et al. [180] adapted the Bioplotting device with a piezoelectric transducer (PZT) generating vibrations while plotting PCL. Scaffolds build had a rougher surface and showed better cell adhesion than the ones build with the conventional setup.

RPBOD: Ang et al. [181] adopted an analogous concept of the 3D-Bioplotter™ technology to develop a robotic dispensing system: the rapid prototyping robotic dispensing (RPBOD) system. The setup consists of a one-component pneumatic dispenser.

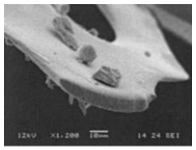
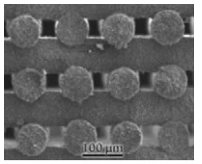
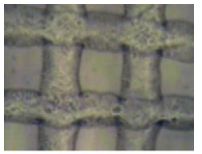
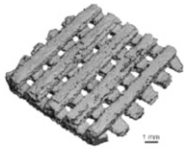
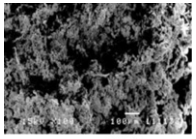
Robocasting: The laminar deposition of highly loaded ceramic slurries (typically 50–65 vol.% ceramic powder) to build a 3D construct using robotics is called robocasting [182]. Unlike the bioplotting process, in most cases the robocasting setup has a stationary dispensing head, while the fabrication platform moves in the planar and vertical field. Inks used for robocasting have to

Table 5
General properties summary of hydrogel compatible RP techniques.

Technique		Drawbacks	Resolution	Porosity	Example[a]	Reference
LASER-BASED SYSTEMS	SLA	Scaffold shrinkage due to water evaporation	30 μm	<90%	 source: [71]	[71,134]
		Incomplete conversion thus post-curing essential				[132]
		Limited resolution				[139]
		Limited availability of cells, Non-homogeneous cell distributions, Cytotoxic photoinitiator				[138]
		Complex architectures with tunable micro- and macroscale features are difficult to achieve				[161]
	μ-SLA	Lower resolution than 2PP	1 μm	<90%	 source: [140]	[141]
		Complex architectures with tunable micro- and macroscale features are difficult to achieve				[161]
		Post-curing can be necessary				
	2PP	Not feasible to produce large scaffolds	< Diffraction limit of applied light	Not specified	 source: [165]	[134]
		Time consuming adjustment to new materials				[95,221]
		Scaffold shrinkage due to water evaporation				[164]
		Diffusion driven polymerization is possible resulting in cross-linking of non-irradiated material				[164]
NOZZLE-BASED SYSTEMS	3D BIOPLOTTER 3D BIOASSEMBLY TOOL 3D CELL ASSEMBLER	Low mechanical strength	45–1600 μm	< 45–60%	 source: [212]	[44,179,213]
		Smooth surface				
		Low accuracy				
		Slow processing				
		Precise control of properties of materials and medium (i.e. mechanical properties of the solution must be high enough to form the 3D structure)				
		Calibration for new material				
		Fused horizontal pores mentioned in some cases				
	RPBOD	Idem 3D-Bioplotter	not specified	not specified	 source: [182]	[181]
		Precise control properties of material and medium				
		Requires freeze drying				
	DIW	Low mechanical strength	5–100 μm	< 90%	 source: [238]	[238,239]
		Smooth surface				
		Low accuracy				
		Slow processing				
		Precise control of properties of materials and medium				

(*continued on next page*)

Table 5 (*continued*)

Technique		Drawbacks	Resolution	Porosity	Example[a]	Reference
	(M)-LDM	Solvent is used Requires freeze drying	300–500 μm	75–90%	 source: [168]	[174,240]
	PAM	Small nozzle inhibits incorporation of particle Narrow range of printable viscosities Solvent is used Highly water-soluble materials cannot be used	7–500 mm	71–94%	 source: [172]	[170]
	ROBOCASTING	Precise control of ink properties is crucial	100–150 mm	< 45%	 source: [184]	[241]
	EXTRUSION/ASPIRATION PATTERNING SYSTEM	A small thermal hysteresis of the products is required Limited applicability	141–300 mm	Not specified	 source: [191]	[241]
PRINTER-BASED SYSTEMS	3DP INKJET PRINTING	Mechanical strength: post-processing often necessary Powder entrapment Availability in powder form	100 μm	33–60%	 source: [222]	[179,221,222,235] [18] [221,222,226,229,235]
		Pore size: dependent on powder particle size Binder droplet size and accuracy of drop placement (resolution of the machine) Clogging of small binder jets	50–250 μm Droplet volume: 80–130 pL		 source: [235]	[17,233,234] [218,222,226–228,230] [218]
		Indirect methods needed (sacrificial molds)	200–500 μm	50%	 source: [232]	[231,232]

[a] All example figures are reprinted with permission from the respective publishers as indicated in the source.

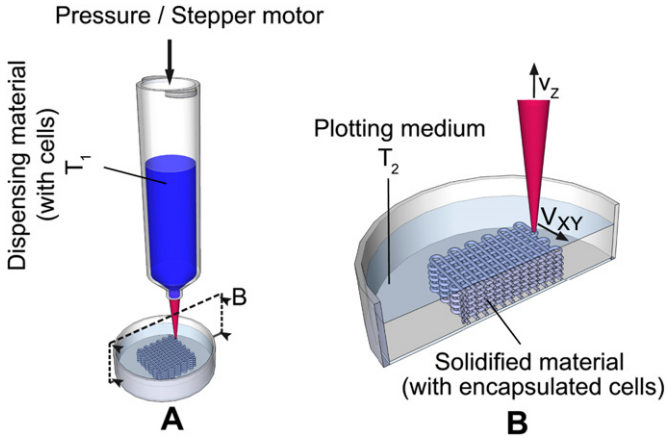


Fig. 5. Scheme of 3D-Bioplotter™ dispensing principle. In the 3D-Bioplotter™ system, the nozzle works pneumatically or via volume-driven injection. This also illustrates the principle of nozzle-based systems in general, where a nozzle is used for the deposition of material. Key difference with other nozzle-based systems is the ability to plot into a liquid medium with matching density, thus introducing buoyancy compensation.

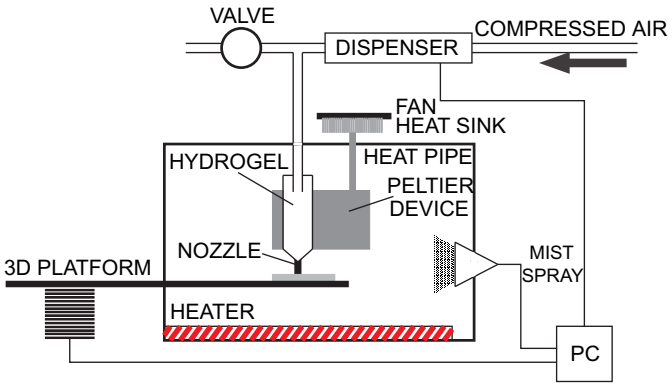


Fig. 6. Schematic illustration of an extruding/aspiration patterning setup. The main difference with other nozzle-based systems is established through the incorporation of a mist spray, thus preventing desiccation of the fabricated constructs.

flow under stress and recover enough stiffness such that, when the stress is released, they can bear both the extruded filament weight and the weight of successive layers [183,184]. Thus, in contrary to other SFF techniques, the concept of robocasting relies essentially on the rheology of the slurry and also on the partial drying of the deposited layers. Building-up constructs from hydrogel material alone is therefore not possible using this technique.

Direct ink writing (DIW): Direct ink writing (DIW) or direct write assembly enables a wide variety of materials to be patterned in nearly arbitrary shapes and dimensions [185–187]. Colloidal-based inks are patterned in both planar and 3D forms with lateral feature sizes that are at least an order of magnitude smaller than those achieved by inkjet printing and other rapid prototyping approaches [188]. The colloidal gels are housed in individual syringes mounted on the *z*-axis motion stage and deposited through a cylindrical nozzle (diameter ranging from 100 μm to 1 mm) onto a moving XY stage. The inks used in this technique must meet two important criteria. First, they must exhibit a well-controlled viscoelastic response, i.e., they flow though the deposition nozzle and then 'set' immediately to facilitate shape retention of the deposited features even as it spans gaps in the underlying layer(s). Second, they must contain a high colloid-volume fraction to minimize drying-induced shrinkage after assembly is complete, i.e., the particle network is able to resist compressive stresses arising from capillary tension [189].

Extruding/aspiration patterning system: The extruding/aspiration patterning system offers both extrusion, and aspiration on the basis of Bernoulli suction. The extrusion and aspiration modes are easily switchable, leading to a variety of applications in cell patterning by combining extrusion/aspiration and using a thermo-reversible hydrogel [190]. This fabrication method makes it possible to produce cell-loaded scaffolds. One of the advantages is the possibility that cell patterns can be filled into another cell matrix. A schematic illustration of an extruding/aspiration patterning system is given in Fig. 6. Nozzle and substrate are placed in a space that controls temperature and humidity. The nozzle is connected to the dispenser, which regulates the pressure of the compressed air, and the valve. The system operates in three modes: extrusion, aspiration, and refilling mode. Closing or opening the valve enables switching between the modes. In a closed configuration, compressed air is supplied to the nozzle and the hydrogel is extruded onto the substrate, or refilled into the aspirated groove. By opening the valve, the compressed air is directed through the valve, and the hydrogel solution is aspirated by Bernoulli suction. To prevent desiccating (to maintain the viability of the cells), a mist spray is used to create a supersaturated atmosphere inside the box.

The most recent trend is the use of hydrogel systems and cell encapsulation strategies to fabricate gel/cell hybrid constructs [191–197]. The 3D-Bioplotter and other similar techniques can be seen as the most straightforward hydrogel/cell manufacturing method for designing complex inner and outer architectures, without the need for an additional support structure. In some cases, the developed machine contains multiple nozzles with different designs (sometimes even printer-based). In literature, 'Cell Assembler' and 'Bioassembly Tool' are common machine names that have a similar working principle. Work of Chang et al. [198] examined the effect of nozzle pressure and size on cell survival and functionality. A quantifiable loss in cell viability was seen, which was caused by a process-induced mechanical damage to cell membrane integrity. It was suggested by the authors that cells might require a recovery period after manufacturing.

3.2.2. Current limitations and hydrogel feasibility for nozzle-based systems

An overview of the combination nozzle-based systems/hydrogel materials is summarized in Table 2. Lately, cell encapsulation strategies have been frequently applied to fabricate gel/cell hybrid constructs [191–195]. For each hydrogel material, it is indicated whether or not cell encapsulation experiments were performed. In addition, Table 5 comprises the more technical details, focused on the disadvantages specific for hydrogels. As shown, scaffolds produced by a 3D-Bioplotter have limited resolution and mechanical strength. Material rigidity was shown to influence cell spreading and migration speed, as demonstrated by Wong et al. [199]. Cells displayed a preference for stiffer regions, and tended to migrate faster on surfaces with lower compliance. In addition, the 3D-Bioplotter technology is a time consuming technique due to the optimization of the plotting conditions for each different material. Ang et al. [181] reported 3D chitosan and chitosan-HA scaffolds using the RPBOD. Solutions of chitosan or chitosan-HA were extruded into a sodium hydroxide and ethanol medium to induce precipitation of the chitosan component. The concentration of sodium hydroxide was identified as important in controlling the adhesion between the layers. The scaffolds were then hydrated, frozen and freeze–dried. Prior to cell culturing with osteogenic cells, the scaffolds were seeded with fibrin glue. Drawbacks of this technique largely follow those of the 3D-Bioplotter and analogues. The inks used in the direct ink writing (DIW) have the disadvantage that the used hydrogel systems must satisfy the two important criteria as mentioned previously.

Khalil et al. [166] developed a multi-nozzle low-temperature deposition system with four different micro-nozzles: pneumatic microvalve, piezoelectric nozzle, solenoid valve and precision extrusion deposition (PED) nozzle. The system consisted of an air pressure supply. Multiple pneumatic valves were simultaneously operated for performing heterogeneous deposition in the development of the 3D scaffold. With this technique, multi-layered cell-hydrogel composites can be fabricated [194]. Hydrogels have also been processed with the PAM technique [172,173]. Of the 3D rapid prototyping micro-fabrication methods available for tissue engineering, PAM has the highest lateral resolution. Recently, it has been demonstrated that the performance of this method is comparable to that of soft lithography [200]. However, capillaries with a very small diameter require careful handling to avoid any tip breakage. In addition, pressures are needed to expel the material from a small orifice. Robocasting relies on the rheology of the slurry and partial drying of the deposited layers. This implies that a pure hydrogel composition cannot be processed via this particular technique, being the most fundamental drawback of the technique. A last nozzle-based system is the extruding/aspiration patterning system. One of the advantages is that its setup is favourable for cell encapsulation purposes and the fact that cell patterns can be filled into another cell matrix. However, the hydrogel materials require a small temperature hysteresis, so it has limited applicability.

Concerning the nozzle-based systems in rapid prototyping of hydrogels, several challenges need to be addressed. Looking at the limited range of materials, the following topics should be addressed: optimal scaffold design, bioactivity of the scaffold as well as the issues of cell seeding and encapsulation possibilities. So, future development will need to focus on the engineering of new materials, the scaffold design and the input of cell biologists. Keeping this in mind, rapid prototyping still remains a promising technique as a methodical interface between tissue and engineering.

Concerning (non home-made) RP devices in general, high equipment purchase costs can be considered a substantial disadvantage. Therefore, it is also noteworthy to mention that very recently open-source low-budget nozzle-based systems have found their way to this research domain. A hydrogel compatible example is the Fab@Home ($1000–3000) system that was developed at Cornell University. Cohen et al. [201] tested a proof-of-concept for *in situ* repair of osteochondral defects using alginate as scaffolding material. Lixandrao et al. [202] demonstrated the feasibility of complex architecture scaffolding with the Fab@Home. They constructed aortic valves based on a PEG-DA/alginate blend.

3.3. Printer-based systems

3.3.1. Working principle and recent trends of printer-based systems

In literature, 'printing' is often used as a general term for both the construction of a scaffold or to indicate printer-based systems. To differentiate between both, we define the latter as manufacturing techniques that implement inkjet technology. Inkjet printers can be divided in drop-on-demand or continuous ejection types. In drop-on-demand systems, electrical signals are used to control the ejection of an individual droplet. In continuous-drop systems, ink emerges continuously from a nozzle under pressure. The jet then breaks up into a train of droplets whose direction is controlled by electrical signals [218]. Both drop-on-demand and continuous-jet systems can be operated with droplets ranging in size from 15 to several hundred microns [218]. Many commercial (adapted) printers fall in the former category, and will only eject ink when receiving a demand signal from the computer. Table 3 classifies the existing inkjet printers (modified from Nakamura et al. [219]). Like the nozzle-based systems, building a construct occurs in an additional computer-controlled layer-by-layer sequence with deposition of material.

3DP™: Prof. Sachs from the Massachusetts Institute of Technology (MIT) introduced the 3D Printing™ technology [220]. It is an example of a solid-phase RP technology. 3D Printing can be used to fabricate parts in a wide variety of materials, including ceramic, metal, metal-ceramic composite and polymeric materials. 3D printing is the only of the solid-phase RP techniques compatible with hydrogel manufacturing. A scheme of a typical 3DP™ setup is given in Fig. 7. The technique employs conventional inkjet technology. The workflow can be described in 3 sequential steps: (1) the powder supply system platform is lifted and the fabrication platform is lowered one layer; (2) the roller spreads the polymer powder into a thin layer (excess powder falls in an overflow vat), and; (3) an inkjet print head prints a liquid binder that bonds the adjacent powder particles together. The binder can dissolve or swell the powder particles, causing bonding via the inter-diffusion of polymer chains or via infiltration of the binder into the powder [221]. Cycling steps 1–3 fabricates a 3D object. A key requirement for 3D printing is the availability of biocompatible powder-binder systems [17]. The powder utilized can be a pure powder or surface-coated powder, depending on the application of the scaffold. It is possible to use a single, one-component powder, or a mixture of different powders blended together [18]. After the finished construct is retrieved, any unbound powder is removed, resulting in a complex three-dimensional part. Basic requirements that the binder system must satisfy: (i) the binder solution must have a high binder content while still having a low viscosity so that it is capable of being deposited by the print head; (ii) minimal conductivity may be required for continuous-jet printing heads; (iii) the binder must dry or cure rapidly so that the next layer of particles can be spread [218].

3D Printing has four steps that can limit the rate of the process: the application of the powder layers, the printing of the binder, the infiltration of the binder into the powder and the drying of the binder [218]. An important advantage of powder-based systems is the production of rougher surfaces, which may enhance cell adhesion [17].

Zcorp developed a 3D printer (Z402) that uses natural polymers as well as plaster of Paris in combination with a water-based ink [221,222]. This opens perspectives towards hydrogel manufacturing. A recent detailed review on 3DP™ concerning all process development steps, such as powder-binder selection and interaction, is given by Utela et al. [223].

In 3DP, control over the geometry is realized by two distinct issues: the minimum attainable feature size, and the variability of part dimensions [218]. Both depend strongly on the binder

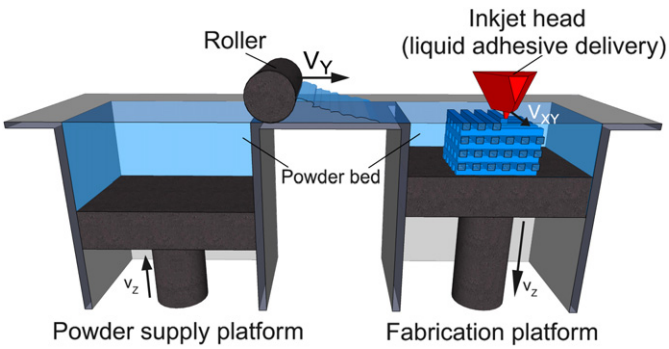


Fig. 7. Schematic representation of a typical 3DP™ setup. A roller spreads a thin layer of polymer powder over the previously formed layer, and is subsequently solidified by the spatially controlled delivery of a liquid binder.

droplet–powder particle interactions. Factors controlling the interaction of powder and binder include: powder material, powder surface treatment, powder size and size distribution, powder shape, powder packing density, binder material, binder viscosity, binder surface tension, droplet size, droplet velocity, temperature of the powder and binder, and ambient temperature [218]. Factors that determine the final object dimensions are: local and accumulative accuracy of deposited layer thickness, accuracy of drop placement, reproducibility of the spread of the printed droplets, and reproducibility of the dimensional changes that accompany binder cure. Sometimes, resolution of the machine is mentioned. Resolution in this context refers to the smallest pores and the thinnest material structures that are obtainable with the equipment [218].

Inkjet printing: This printer-based subclass comprises all liquid-phase inkjet technologies. It can vary from setups similar to the 3DP™ system in which the powder bed is replaced by a liquid hydrogel precursor [224], or systems that use direct inkjet writing [13,225]. In the case of direct inkjet writing, the construct is build-up by the deposited liquid itself.

3.3.2. Current limitations and hydrogel feasibility for printer-based systems

Printer-based systems can perhaps be regarded as the least hydrogel/cell suitable of the systems that allow hydrogel processing. Tables 4 and 5, summarize the hydrogel feasibility respectively limitations towards hydrogel manufacturing. Wu et al. [226] described the use of polyethylene oxide (PEO) and poly-ε-caprolactone (PCL) as matrix materials and a 20% PCL-LPS/chloroform binder solution to create a 3D device for controlled drug release. Top and bottom layer of the tabular device was made out of slowly degrading PCL, while the interior layers were composed of PEO bound by printing binder solutions. The local microstructure of the device could be controlled by either changing the binder or by changing the printing parameters (velocity). Typical powder particle size ranged from 45 to 75 μm for PCL and 75–150 μm for PEO. The binder droplets had a diameter in the order of 60–80 μm. After 20 h, significant swelling of the PEO was experimentally observed.

Landers et al. [179] studied the use of water-soluble polymers, which are bonded together by means of water-based saccharide glues. Although the choice of powders and the corresponding adhesives appears to be unlimited, this technology requires post-sintering or post-curing to improve mechanical as well as environmental stability.

Lam et al. [222] developed a blend of starch-based powder containing cornstarch (50 wt.%), dextran (30 wt.%) and gelatin (20 wt.%). Distilled water was used as a suitable binder material. Cylindrical scaffolds (Ø 12.5 × 12.5 mm) were produced having either cylindrical (Ø 2.5 mm) or rectangular (2.5 × 2.5 mm) pores. Using water as the binder means that the problem of a toxic fabrication environment was eliminated and the problem of residual solvent in the construct was solved. Other advantages of using a water-based binder include the possibility to incorporate biological agents (e.g. growth factors) or living cells. Post-processing of the scaffolds was necessary to enhance the strength of the scaffolds and increase the resistance against water uptake. The scaffolds were dried at 100 °C for 1 h after printing and infiltrated with different amounts of a copolymer solution consisting of 75% poly(L-lactide) and 25% PCL in dichloromethane.

Sanjana et al. [227] reported on the use of inkjet printing to fabricate neuron-adhesive patterns such as islands and other shapes using poly(ethylene) glycol (PEG) as cell-repulsive material and a collagen/poly-D-lysine (PDL) mixture as cell-adhesive material. They worked with a positive relief: PEG used as background and anti-fouling material was bonded covalently to the glass surface while the collagen/PDL mixture was used as the printed foreground and cell-adhesive material. They also suggest that the inkjet printing technique could be extrapolated to building 3D structures in a layer-by-layer fashion.

Xu et al. [228] use the inkjet printing technology for the construction of three-dimensional constructs, based on fibrin gel. Fibrin was used as a printable hydrogel to build 3D neural constructs. The fibrin is formed by the enzymatic polymerization of fibrinogen by addition of thrombin and $CaCl_2$. First, a thin sheet of fibrinogen was plated and subsequently, thrombin droplets were ejected from the print cartridge onto the pre-plated fibrinogen layer. Fibrin gel formation was observed immediately after thrombin ejection. Subsequently, NT2 neurons were printed on the gelled fibrin. The whole procedure was repeated 5 times, resulting in a 3D neural sheet.

Koegler et al. [229] described the fabrication of 3DP scaffolds based on poly(L-lactide-co-glycolide). Surface chemistry of these scaffolds was modified by reprinting the top surface with a solution of Pluronic F127 in $CHCl_3$.

Cui et al. [230] reported on the fabrication of micron-sized fibrin channels using a drop-on-demand polymerization. A thrombin/Ca^{2+} solution together with human micro-vascular endothelial cells (HMVEC) cells was used as 'bio-ink' and sprayed by the inkjet technology onto a fibrinogen substrate. They suggested that these constructs show potential in building complex 3D structures. Examples of the direct use of printer-based systems together with hydrogels are rather limited. In some cases, the use of an indirect system is mentioned.

Sachlos et al. [231] use an indirect approach to produce collagen scaffolds with predefined and reproducible complex internal morphology and macroscopic shape by developing a sacrificial mould, using 3D printing technology. This mould is then filled with a collagen dispersion and frozen. The mould is subsequently removed by chemical dissolution in ethanol and a solid collagen scaffold was produced using critical point drying.

Yeong et al. [232] also utilized a similar indirect approach to fabricate collagen scaffolds. In addition, they investigated different drying routes after removal of the sacrificial mould with ethanol. The effects of a freeze drying process after immersion of the scaffold in distilled water and critical point drying with CO_2 reflected onto dimensional shrinkage, pore size distribution and morphology in general.

Boland et al. [224] described the use of the inkjet printing technique for the construction of synthetic biodegradable scaffolds. They used a 2% alginic acid solution, a liquid that is known to cross-link under mild conditions to form a biodegradable hydrogel scaffold. The ink cartridge was filled with 0.25 M calcium chloride ($CaCl_2$), which is known to promote the cross-linking of the individual negatively charged alginic acid chains resulting in a 3D network structure. This cross-linker was printed onto liquid alginate/gelatin solutions.

The biggest obstacles for RP technologies, thus also printer-based systems, are the restrictions set by material selection and aspects concerning the design of the scaffold's inner architecture. Thus, any future development in the RP field should be based on these biomaterial requirements, and it should concentrate on the design of new materials and optimal scaffold design [18]. The selected scaffold material must be biocompatible, compatible with the printing process, and it must be easily manufactured in the form required (powder or liquid) [233]. In the case of powder material, the particle size must be controllable. Another issue is the sterility of the manufacturing process and products and their ability to withstand sterilization processes [17]. Off course, this plays a pivotal role for all systems when embedding cells during the process.

Some limitations are caused by material trapped in small internal holes. These trapped liquid or loose powder materials may be difficult or even impossible to remove afterwards, and in some cases, these residues may even be harmful to cells and tissues. Experimental results show that the smoother the surface generated, the easier the removal of trapped material [17,18]. Smoother surfaces are on the other hand less desirable for cell adhesion purposes.

Limitations of 3DP include the fact that the pore sizes of fabricated scaffolds are dependent on the powder size of the stock material. As such, the pore sizes available are limited to smaller pore values (<50 μm) widely distributed throughout the scaffold. More consistent pore sizes, including larger pores, can be generated by mixing porogens (of pre-determined sizes) into the powder prior to scaffold fabrication. However, incomplete removal of the porogens is sometimes observed due to incomplete leaching. The mechanical properties and accuracy of 3DP fabricated scaffolds are other considerations that need to be addressed [234].

Despite the idea of using a water-based ink in order to eliminate a toxic fabrication environment, and thus creating an opportunity to incorporate biological agents or even living cells, toxic post-processing of the constructs is often needed to improve the mechanical properties. Suwanprateeb [235] described a double infiltration technique to increase the mechanical properties of natural polymers fabricated by three-dimensional printing using a water-based binder. The 3DP parts were porous in nature since the powder bed was only lightly packed during the process and only the surface of the powder granules was connected by a binder. Porosity typically ranged between 50 and 60%. To enhance the performance of the 3DP parts based on a mixture of 40 wt.% starch, 15 wt.% cellulose fibre, 25 wt.% sucrose sugar and 20 wt.% maltodextrin, infiltration by some other material was performed. The infiltration material used in this experiment was a heat-cured dental acrylate prepared by mixing triethylene glycol dimethacrylate, 2,2-bis[4(2-hydroxy-3-methacryloyloxypropyloxy)-phenyl] propane and a polyurethane dimethacrylate in a 40:40:20 wt.% proportion. Benzoylperoxide was used as initiator. After infiltration, the specimen was cured at 105 °C for 30 min. From the results, it was found that double infiltration and curing of 3DP samples increased the performance of specimens in wet conditions.

Pfister et al. [221] described the fabrication of biodegradable polyurethane scaffolds by 3D Printing. In this case, the polyurethane formation is the post-treatment step. Commercially available powder ZP11 (a powder blend of starch, short cellulose fibres and dextrose as a binder) was processed by the printing of an aqueous ink, which activates the dextrose binder. The resulting objects were very fragile and highly water-soluble. Therefore, the authors selected an additional post-treatment step involving infiltration and partial cross-linking with lysine ethylester diisocyanate. The starch polyols react with the isocyanate to form network structures. The obtained structures exhibited much improved mechanical stability. Because of the presence of starch incorporated in the network, the structures could swell and the resulting lysine-based polyurethane networks were biodegradable upon exposure to water and body fluids.

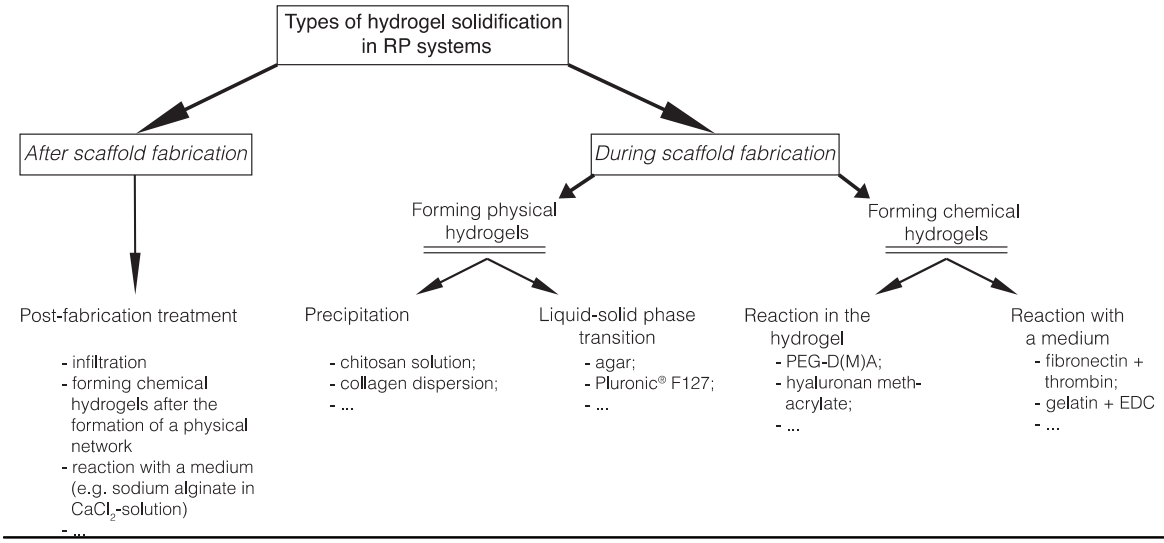
3.4. Preserving the mechanical integrity of RP processed hydrogels

As previously mentioned, the mechanical properties of a certain biomaterial play a partial, although crucial role in its potential success as a scaffolding material. More specific, it has been generally accepted that in designing a proper scaffold biomaterial, one must strive to translate the mechanical characteristic features of the target tissue into the mechanical features of the fabricated construct. For instance, hard tissue regeneration requires strengths of 10-–1500 MPa, while soft tissue strengths are typically located between 0.4 and 350 MPa [242]. As a part of it, preserving the mechanical integrity of the scaffold contributes substantially to the completion of this demand. The latter appears to be of utmost importance with respect to hydrogels and will for that reason be discussed in this subsection. Moreover, it is possible to enhance the control over not only the mechanical properties, but even the biological effects and degradation kinetics by hierarchical design of scaffolds with micron to millimeter features [234]. In the case of hydrogel performance, degradation rates are controlled by hydrolysis, enzymatic reactions or simply by dissolution of the matrix (e.g. ion exchange in Ca^{2+} cross-linked alginate systems).

The process of obtaining a construct with suitable strength starts with the solidification and simultaneous shaping of the material in a certain pattern. An overview summarizing the most frequently applied and different solidification mechanisms is given in Table 6. Although our discussion focuses on hydrogel materials, other materials could easily be incorporated into this scheme.

The application of natural as well as synthetic hydrogels in TE and as cell embedding materials has been a review topic of several

Table 6
Classification of the most frequently applied hydrogel solidification mechanisms in RP processing.



authors [12,26,33,66]. The solidification or gelling mechanisms of hydrogels include inherent phase transition behaviour and cross-linking (ionic or covalent) approaches. Regarding the former mechanism, careful control over the printing temperatures can provide for some hydrogels a phase transition from solution to gel state, in particular for polymers with a lower critical solution temperature (LCST) behaviour (e.g. Pluronic® F127). However, this behaviour is reversible. The formation of an ionically cross-linked network through the use of multivalent counterions, e.g. sodium alginate and Ca^{2+} ions, provides more control over the mechanical integrity. Nevertheless, these ions could be leached out in long-term culture, or even be exchanged by other ionic molecules, compromising the control over the construct properties. Therefore, in most cases, covalent network formation is required in order to precisely enhance the mechanical stability and reproducibility of the constructs. The light-induced radical cross-linking of monomers and/or prepolymer solutions have established a quasi monopoly as a hydrogel solidification strategy in combination with RP fabrication schemes. In the case of the laser-based systems, this is even the fundament on which the respective techniques are based and those are striking examples of one-step cross-linking approaches. The chemical structures of some well-known photoinitiators are shown in Fig. 8. The first structure (Fig. 8(A)) is an example of a D-π- chromophore, known for its high sensitivity in 2PP processes [243,244]. The π-part is a conjugated backbone symmetrically substituted at the ends by electron-donating D-parts. Fig. 8(B) represents the chemical formula of Irgacure® 2959 (I2959), a commonly applied photoinitiator thanks to its high biocompatibility. It is known to absorb light in the UV range. The last structure represents camphorquinone (CQ; Fig. 8(C)), an initiator with many dental applications and visible light working range.

Different reactive thermo-sensitive thiolated systems were developed in the past [245–252]. Thiol, vinyl or allyl ether polymerization display some advantages: mild reaction conditions, photoinitiator not necessary, low or even no oxygen inhibition effects, fast process, forming of cross-linked networks with good physical and mechanical properties [253] and degradable in (mimicked) physiological conditions [254].

The so-called 'tandem reaction' consists of two steps. The first step is the occurrence of rapid gelation kinetics. The second step involves a covalent curing based on the Michael-type addition. This technique allows to create covalently bonded hydrogels in combination with functional cells under physiological conditions, with no side reactions with bioactive molecules [255].

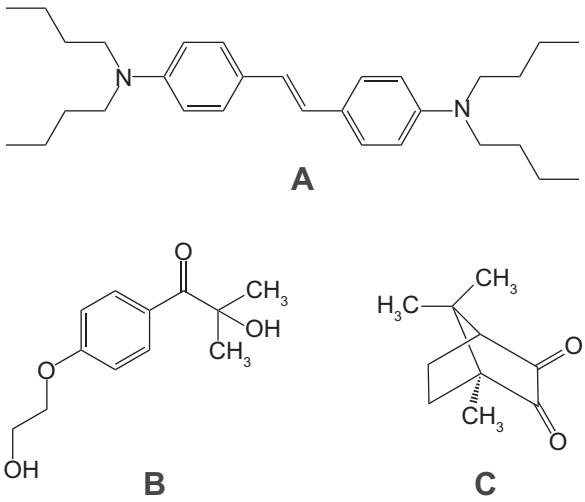


Fig. 8. Some typical photoinitiators used for (A) 2PP – an example of a strongly absorbing two photon absorbing D-π-D chromophore –, (B) general UV curing – Irgacure® 2959 –, and (C) visible light curing – camphorquinone –.

When performing cross-linking in general, be it radical (light, redox, temperature induced) or non-radical (enzymatic, ionic, carbodiimide, glutaraldehyde, genipin,…), the toxicity of the cross-linkers should be carefully considered. This aspect is of crucial importance towards the success of a construct. For instance, comparing the biocompatibility of genipin and glutaraldehyde as a way to non-radically cross-link gelatin demonstrated the cytotoxic effect of glutaraldehyde and to a lower extent that of genipin [34,192]. A possible aldol condensation cross-linking mechanism for genipin was proposed by Liang et al. [256] and the authors also provided evidence for its better biocompatibility compared to carbodiimide cross-linking with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC). However, genepin has only few applications because of its high cost, and dark blue staining, which could interfere with cell characterization techniques [192]. Thrombin, on the other hand, did not show any substantial adverse effects [192,204]. Next to those non-radical cross-linking mechanisms, radical cross-linking induced through light irradiation is accepted as a common strategy. Cytotoxicity studies of several frequently used UV and visible light initiating systems have been performed [257,258]. CQ at concentrations ≤0.01% (w/w) and low intensity irradiation (~60 mW cm^{-2}; 470–490 nm) appeared to have less toxic effects than isopropyl thioxanthone (ITX) as visible light initiator. Another promising visible light initiator that absorbs at 512 nm is eosin Y [259,260], although up to date no relevant cytotoxicity studies have been performed. Out of three different Irgacure® initiators, it was the I2959 initiating system (~6 mW cm^{-2}; 365 nm) that held the greatest potential at concentrations below 0.05% (w/w). Additionally, it was also found that those cytotoxic effects varied depending on the cell types [258,261]. Performing radical cross-linking reactions without the use of either visible or UV light can also be mediated through the use of redox systems. Duan et al. [262] reported on the negative cooperative effect of a water-soluble redox initiating system, consisting out of ammonium persulfate (APS) and N, N, N′, N′-tetramethylethylenediamine (TEMED), on NIH/3T3 fibroblasts. The system was used to trigger PEG-DA polymerization. Redox initiating systems have been extensively reviewed before [263].

In other cases, the formation of chemical hydrogels can only be attained after scaffold fabrication. Post-fabrication UV curing of a physically cross-linked photosensitive Pluronic F127-Ala-L construct with Irgacure® 2959 as initiator was for instance performed by Fedorovitch et al. [213] in order to acquire a chemical network. This example directly serves as an easy template into attaining multiple-step cross-linking. When working with blends of different hydrogels and combinations of physical as well as chemical network formations, multiple-step cross-linking systems of higher complexity are needed. Very recently, Xu et al. [196] demonstrated a fascinating state-of-the-art example of such an approach by blending gelatin, sodium alginate and fibrinogen with cells and stepwise cross-linking of the sodium alginate with a $CaCl_2$ solution and the fibrinogen with a thrombin solution. The gelatin component served as a necessary co-material blend in to provide sol to gel phase transition during the fabrication process [192]. Other examples can be found in the work of Xu et al. [204], Zhang et al. [203], Skardal et al. [197] and, Yan et al. [205].

4. Fundamentals on rapid prototyping in scaffold-free tissue engineering

In general, the application of scaffolds in a TE approach is straightforward, but still subject to some challenges [264,265]. These can be divided in two distinct categories: (i) complications

posed by host acceptance (immunogenicity, inflammatory response, mechanical mismatch), and; (ii) problems related to cell cultures (cell density, multiple cell types, specific localization). It is surprisingly interesting that, during embryonic maturation, tissues and organs are formed without the need for any solid scaffolds [266]. The formation of a final pattern or structure without externally applied interventions, in other words the autonomous organization of components, is called self-assembly [267,268]. A premise concerning the self-assembly and self-organizing capabilities of cells and tissues is worked out in the field of scaffold-free TE. This idea poses an answer to the immunogenic reactions and other unforeseen complications elicited through the use of scaffolds. One way of implementing this self-assembly concept is the use of cell sheet technology, which was demonstrated by L'Heureux and colleagues for the fabrication of vascular grafts [269,270]. In a similar way, the group of Okano engineered long-lasting cardiac tissue based on cell sheet strategies [271–273]. Remarkably, some have already reached clinical trials [273,274]. An alternate approach was selected by McGuigan and Sefton, who encapsulated HepG2 cells in cylindrical sub-millimetre gelatin modules, followed by endothelializing the surfaces [34]. A construct with interconnected channels that enabled perfusion was generated through random self-assembly of the cell/hydrogel modules. However, the implementation of RP technologies offers another even more fascinating perspective on scaffold-free TE, and is commonly termed 'bioprinting' or 'organ printing'.

We define organ printing as the engineering of three-dimensional living structures supported by the self-assembly and self-organizing capabilities of cells delivered through the application of RP techniques based on either laser [275–278], inkjet [219,228,230,236,279–284], or extrusion/deposition [193,197,285–290] technology. An emerging laser-based RP technique called biological laser printing (BioLP) stems from an improved matrix assisted pulsed laser evaporation direct write (MAPLE DW) system. The improvement is realized by incorporation of a laser absorption layer and thus eliminating the direct interaction with the biological materials. The principle is illustrated in Fig. 9. Prior to laser exposure, a cell suspension layer is formed on top of the absorption layer. Then, a laser beam is focused on the interface of the target, which causes a thermal and/or photomechanical ejection of the cell suspension towards the substrate [275]. Target and substrate are both able to move in the planar field.

The workflow of inkjet or extrusion-based bioprinting can be represented by Fig. 10. In short, balls of bio-ink are deposited in well-defined topological patterns into bio-paper sheets. The bio-ink building blocks typically have a spherical or cylindrical shape, and consist of single or multiple cell types. Several bio-ink

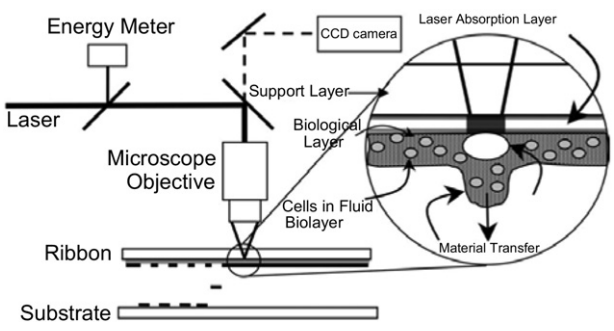


Fig. 9. Schematic illustration explaining the working principle of BioLP. A focussed laser beam initiates material transfer towards the substrate. Interestingly, a laser absorption layer prevents direct interaction between the laser and the biological materials. The scheme was reused from [277] with the permission from Springer.

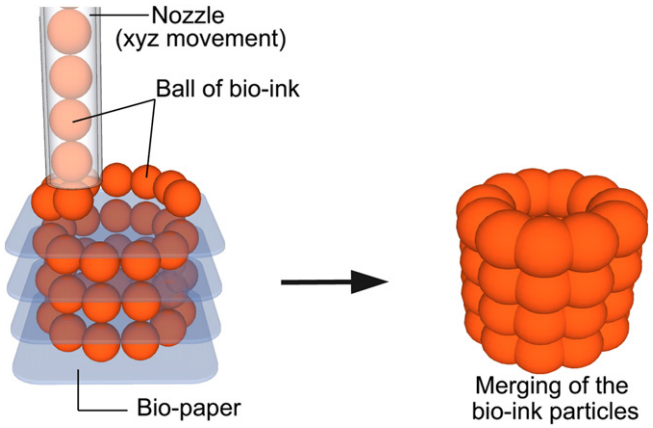


Fig. 10. Basic concept of bioprinting bio-ink particles into bio-paper (hydrogel) sheets. The bio-ink particles are deposited in a tubular geometry (left). After the deposition is finished, the construct is transferred to a bioreactor to fuse the bio-particles and further maturation made possible (right).

preparation methods have been described [264,266,285,289]. In a post-processing step, the construct is transferred to a bioreactor and the bio-ink spheres are fused. The bio-paper, a hydrogel, can be removed after construction if required. Bio-printers can either have a jet design or work like a mechanical extruder [102,291]. This implies that several RP apparatuses described in the previous part can serve as a bio-printer (e.g. the Bioassembly Tool, 3D-Bioplotter™,…), if sterile conditions can be acquired. In the case of inkjet technology, individual or small cell clusters are printed. Despite the advantageous speed, versatility and cost, high cell densities are difficult to obtain and considerable cell damage is induced [264,291]. On the other hand, extrusion-based bio-printers are more expensive but offer a more gentle approach towards cells.

In this context, hydrogels are employed as bio-paper and only provide a temporary support for the deposited bio-ink particles. In other words, the bio-paper is clearly different from scaffolds used in classical scaffold-based TE. Arai et al. [292] made use of a hydrogel consisting of 2.0% $CaCl_2$, 20 wt.% PVA and 3wt.% hyaluronan for the deposition of alginate based bio-ink particles. In most cases, this bio-paper hydrogel will have a sheet-like design (e.g. Fig. 10). For instance, Boland et al. [236] made use of thermo-sensitive gels to generate sequential layers for cell printing. The group developed a cell printer, derived from commercially available inkjet printers that enable to place cells in positions mimicking their spatial location in an organ [280]. The printer can put up to nine solutions of cells or polymers into a specific place by the use of specially designed software, and print two-dimensional tissue constructs. Extending this technology to three dimensions is performed by the use of thermo-reversible gels. These gels were a fluid at 20 °C and a gel above 32 °C and serve as bio-paper on which tissue structures can be printed. Dropping another layer of gel onto the already printed surface could generate successive layers. The thermo-sensitive gel used for the experiments was a poly [N-isopropylacryamide-co-2-(N,N-dimethylamino)-ethyl acrylate] copolymer in a concentration of 10 wt.% polymer in cold, deionized water.

However, collagen used in a sheet-like design appeared to have integrated into the final structure, posing difficulties in its removal [289]. Depending on the target tissue design, the bio-paper can also have other geometries. For instance, agarose rods were plotted and easily removed after post-printing fusion of a multicellular construct in order to fabricate tubular constructs (Fig. 11) [285]. Agarose, being an inert and biocompatible hydrogel, is not remodelled by the cells and can easily be removed after fusion of

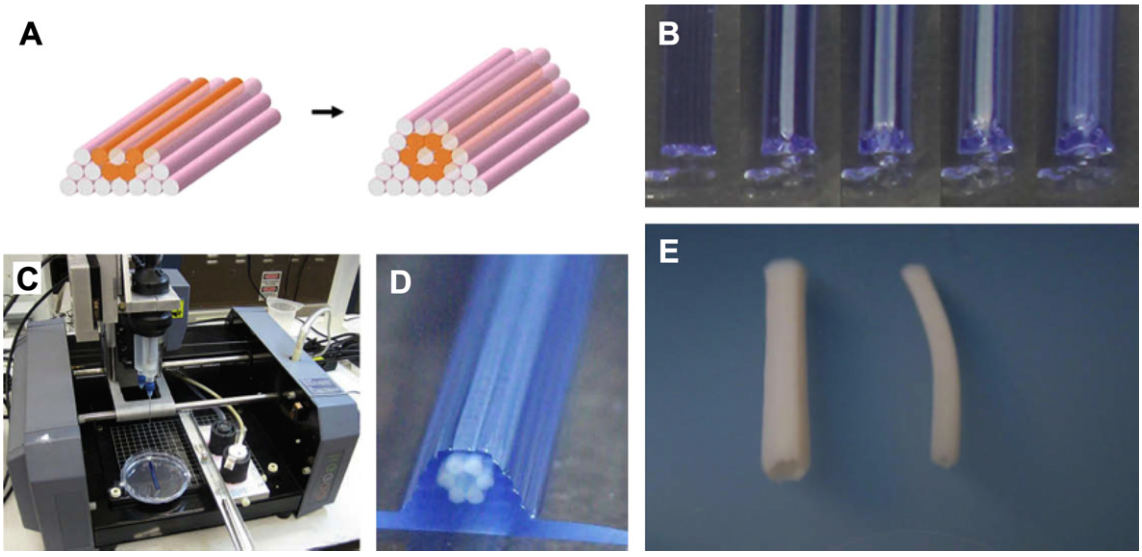


Fig. 11. Bioprinting tubular structures with cellular cylinders. (A) Designed print template (B) Layer-by-layer deposition of agarose (blue) cylinders and multicellular pig SMC cylinders (white). (C) The bio-printer outfitted with two vertically moving print heads. (D) The printed construct. (E) Engineered pig SMC tubes of distinct diameters resulted after 3 days of post-printed fusion (left: 2.5 mm OD; right: 1.5 mm OD). Pictures were reprinted from [287] with permission from Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the bio-ink. *In situ* cross-linkable synthetic ECM (sECM) mimetic hydrogels formed by co-cross-linking PEG-DA and modified hyaluronic acid (HA)/gelatin to the corresponding thiolated dithiopropionylhydrazide (DTPH) derivatives were developed as bio-paper by Mironov et al. [291]. Other examples of hydrogels that served as bio-paper include fibrin, Matrigel™, fibrinogen, PNIPAM and polyethylene glycol tetra-acrylates [230,236,277,286].

It is somewhat unclear as to whether the fabrication of cell/gel hybrid constructs, which has already been brought up in the previous part, falls under the category of bioprinting or if it can be regarded, as it is our understanding, as a bridge between pure scaffold-based and pure scaffold-free TE strategies. For instance, Skardal et al. [197] methacrylated HA and gelatin, in order to fabricate tubular photocross-linkable constructs from partially pre-cross-linked hydrogels and cells suspended within. The importance that is correlated to the biomaterial(s) used, as is clarified in Fig. 12, notes the primary distinction with pure scaffold-free TE. Xu and colleagues made 3D cell/gel hybrid scaffolds from ADS cells and gelatin/alginate/fibrin hydrogels to model the metabolic syndrome (MS) in 3D [196]. Their work revealed the potential use of this 3D physiological model for drug discovery and the use of fibrin as an effective material to regulate ADC cell differentiation and self-organization into adipocytes and endothelial cells. Nevertheless the importance appointed to the biomaterial, cells are being encapsulated within the structure, and therefore this approach deflects somewhat from pure scaffold-based engineering. May be the future shows that this state-of-the-art TE concept and crossover approach of the two main distinct TE premises leads to some fascinating results and research.

5. Future directions

A couple of interesting emerging trends tend to bring scaffold-based TE on the next level. First of all, the implementation of

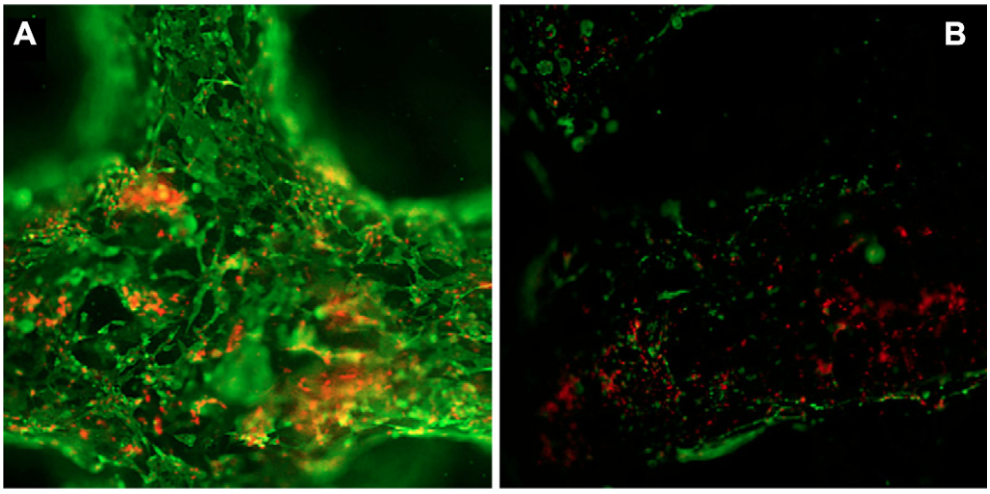


Fig. 12. ADC cells, cultured with EGF, in a 3D gelatin/alginate/fibrin scaffold to differentiate into endothelial cells and adipocytes. (A, B) Immunostaining with mAbs for mature endothelial cells in green and PI for nuclear in red. (A) ADC cells within a gelatin/alginate/fibrin construct, and (B) without fibrin. The pictures were reprinted from [197] with permission from Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

gradient techniques, in the general sense, seems to provide some promising approaches. The utilization of blends layed down the foundation of working with material gradients. Via this way, a more precise mimicking of the ECM composition and mechanical properties will be possible, with spatial alterations throughout the scaffold. Of course, this can be extended towards different and multiple cell types, biomolecules, growth factors, etc., deposited in predefined patterns throughout the scaffold. Furthermore, gradients applied on the deposited scaffold pattern in itself (the deposited strand configuration) offers interesting alternatives to alter the mechanical properties of the construct. Although some authors already performed some initial experiments [167,192], the applicability of such approaches needs to be investigated in depth [167,191].

Second, combining multiple fabrication methods to obtain a single construct appears to be useful. For instance, combining electrospinning (~nanoporosity) and bioplotting (~microporosity) for the production of a single scaffold was demonstrated by Kim et al. [293]. The combined effect of different techniques will most likely exhibit positive cooperative effects. Thus, instead of focussing on the exploitation of one single technique, it would be most fruitful to combine the positive effects of different techniques into one operation procedure.

Last but definitely the most fascinating trend, material scientists should incorporate the knowledge of engineers into the designing step of the construct. This last item is somewhat related to the first one, with a clear, distinct focus on the mechanical properties. By means of finite element modelling, predicting the mechanical properties of the construct can be handful. More importantly, adjusting the (predicted) mechanical properties of a model simply by varying the geometrical design of the material offers an interesting path to match its expected properties and the desired properties. The principles, advantages, and possible applications of this so-called Bio-CAD modelling in TE have been reviewed in 2005 by Sun et al. [224,294]. In the last couple of years, more and more, bone-engineering scientists follow this methodology [295–297]. However, in the case of soft tissue engineering and/or tailored hydrogel scaffolds, this has not yet been intensively explored.

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References

[1] OPTN. Donation and transplantation. Online. Available from URL: http://optn.transplant.hrsa.gov/about/; 2012.
[2] Wolfe RA, Roys EC, Merion RM. Trends in organ donation and transplantation in the United States 1999-2008. Am J Transplant 2010;10:961–72.
[3] Desmet T, Schacht E, Dubruel P. Rapid prototyping as an elegant production tool for polymeric tissue engineering scaffolds: a review. In: Barnes SJ, Harris LP, editors. Tissue engineering: roles, materials and applications. New York: Nova Science Publishers; 2008. p. 141–89.
[4] Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920–6.
[5] Bonassar LJ, Vacanti CA. Tissue engineering: the first decade and beyond. J Cell Biochem; 1998:297–303.
[6] Griffith LG, Naughton G. Tissue engineering - current challenges and expanding opportunities. Science 2002;295:1009–14.
[7] Chen QZ, Harding SE, Ali NN, Lyon AR, Boccaccini AR. Biomaterials in cardiac tissue engineering: ten years of research survey. Mat Sci Eng R-Rep 2008;59:1–37.
[8] Gerlier L, Lamotte M, Wille M, Dubois D. Cost-utility of autologous chondrocytes implantation using chondrocelect (r) in symptomatic knee cartilage damage in belgium. Value Health 2009;12:A443.
[9] Hayashi R, Yamato M, Takayanagi H, Oie Y, Kubota A, Hori Y, et al. Validation system of tissue-engineered epithelial cell sheets for corneal regenerative medicine. Tissue Eng Pt C-Meth 2010;16:553–60.
[10] Asakawa N, Shimizu T, Tsuda Y, Sekiya S, Sasagawa T, Yamato M, et al. Pre-vascularization of in vitro three-dimensional tissues created by cell sheet engineering. Biomaterials 2010;31:3903–9.
[11] Sasagawa T, Shimizu T, Sekiya S, Haraguchi Y, Yamato M, Sawa Y, et al. Design of prevascularized three-dimensional cell-dense tissues using a cell sheet stacking manipulation technology. Biomaterials 2010;31:1646–54.
[12] Barcili B. Hydrogels for tissue engineering and delivery of tissue-inducing substances. J Pharm Sci 2007;96:2197–223.
[13] Ilkhanizadeh S, Teixeira AI, Hermanson O. Inkjet printing of macromolecules on hydrogels to steer neural stem cell differentiation. Biomaterials 2007;28:3936–43.
[14] Chen FM, Shelton RM, Jin Y, Chapple ILC. Localized delivery of growth factors for periodontal tissue regeneration: role, strategies, and perspectives. Med Res Rev 2009;29:472–513.
[15] Ma PX. Scaffolds for tissue fabrication. Mater Today 2004;7:30–40.
[16] Butler DL, Shearn JT, Juncosa N, Dressler MR, Hunter SA. Functional tissue engineering parameters toward designing repair and replacement strategies. Clin Orthop Relat R; 2004:S190–9.
[17] Peltola SM, Melchels FPW, Grijpma DW, Kellomaki M. A review of rapid prototyping techniques for tissue engineering purposes. Ann Med 2008;40:268–80.
[18] Yeong WY, Chua CK, Leong KF, Chandrasekaran M. Rapid prototyping in tissue engineering: challenges and potential. Trends Biotechnol 2004;22:643–52.
[19] Sachlos E, Czernuszka JT. Making tissue engineering scaffolds work. review: the application of solid freeform fabrication technology to the production of tissue engineering scaffolds. Eur Cell Mater 2003;5:29–39.
[20] Martin I, Wendt D, Heberer M. The role of bioreactors in tissue engineering. Trends Biotechnol 2004;22:80–6.
[21] Stephens JS, Cooper JA, Phelan FR, Dunkers JP. Perfusion flow bioreactor for 3d in situ imaging: investigating cell/biomaterials interactions. Biotechnol Bioeng 2007;97:952–61.
[22] Awad HA, Wickham MQ, Leddy HA, Gimble JM, Guilak F. Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. Biomaterials 2004;25:3211–22.
[23] Benoit DSW, Schwartz MP, Durney AR, Anseth KS. Small functional groups for controlled differentiation of hydrogel-encapsulated human mesenchymal stem cells. Nat Mater 2008;7:816–23.
[24] Chenite A, Chaput C, Wang D, Combes C, Buschmann MD, Hoemann CD, et al. Novel injectable neutral solutions of chitosan form biodegradable gels in situ. Biomaterials 2000;21:2155–61.
[25] Elisseeff J, Anseth K, Sims D, McIntosh W, Randolph M, Langer R. Transdermal photopolymerization for minimally invasive implantation. P Natl Acad Sci USA 1999;96:3104–7.
[26] Nicodemus GD, Bryant SJ. Cell encapsulation in biodegradable hydrogels for tissue engineering applications. Tissue Eng Pt B-Rev 2008;14:149–65.
[27] Williams CG, Kim TK, Taboas A, Malik A, Manson P, Elisseeff J. In vitro chondrogenesis of bone marrow-derived mesenchymal stem cells in a photopolymerizing hydrogel. Tissue Eng 2003;9:679–88.
[28] Vinatier C, Magne D, Weiss P, Trojani C, Rochet N, Carle GF, et al. A silanized hydroxypropyl methylcellulose hydrogel for the three-dimensional culture of chondrocytes. Biomaterials 2005;26:6643–51.
[29] Schmedlen KH, Masters KS, West JL. Photocrosslinkable polyvinyl alcohol hydrogels that can be modified with cell adhesion peptides for use in tissue engineering. Biomaterials 2002;23:4325–32.
[30] Masters KS, Shah DN, Leinwand LA, Anseth KS. Crosslinked hyaluronan scaffolds as a biologically active carrier for valvular interstitial cells. Biomaterials 2005;26:2517–25.
[31] Liu VA, Bhatia SN. Three-dimensional photopatterning of hydrogels containing living cells. Biomed Microdevices 2002;4:257–66.
[32] Tsang VL, Chen AA, Cho LM, Jadin KD, Sah RL, DeLong S, et al. Fabrication of 3d hepatic tissues by additive photopatterning of cellular hydrogels. FASEB J 2007;21:790–801.
[33] Hoffman AS. Hydrogels for biomedical applications. Adv Drug Deliver Rev 2002;54:3–12.
[34] McGuigan AP, Sefton MV. Modular tissue engineering: fabrication of a gelatin-based construct. J Tissue Eng Regen M 2007;1:136–45.
[35] van Susante JLC, Buma P, Schuman L, Homminga GN, van den Berg WB, Veth RPH. Resurfacing potential of heterologous chondrocytes suspended in fibrin glue in large full-thickness defects of femoral articular cartilage: an experimental study in the goat. Biomaterials 1999;20:1167–75.
[36] Fussenegger M, Meinhart J, Hobling W, Kullich W, Funk S, Bernatzky G. Stabilized autologous fibrin-chondrocyte constructs for cartilage repair in vivo. Ann Plas Surg 2003;51:493–8.
[37] Matsusaki M, Yoshida H, Akashi M. The construction of 3d-engineered tissues composed of cells and extracellular matrices by hydrogel template approach. Biomaterials 2007;28:2729–37.

[38] Yao R, Zhang RJ, Yan YN, Wang XH. In vitro angiogenesis of 3d tissue engineered adipose tissue. J Bioact Compat Pol 2009;24:5–24.
[39] Bian WN, Bursac N. Engineered skeletal muscle tissue networks with controllable architecture. Biomaterials 2009;30:1401–12.
[40] Bryant SJ, Anseth KS. The effects of scaffold thickness on tissue engineered cartilage in photocrosslinked poly(ethylene oxide) hydrogels. Biomaterials 2001;22:619–26.
[41] Bryant SJ, Nicodemus GD, Villanueva I. Designing 3d photopolymer hydrogels to regulate biomechanical cues and tissue growth for cartilage tissue engineering. Pharm Res 2008;25:2379–86.
[42] Endres M, Hutmacher DW, Salgado AJ, Kaps C, Ringe J, Reis RL, et al. Osteogenic induction of human bone marrow-derived mesenchymal progenitor cells in novel synthetic polymer-hydrogel matrices. Tissue Eng 2003;9: 689–702.
[43] Fedorovich NE, Alblas J, de Wijn JR, Hennink WE, Verbout AJ, Dhert WJA. Hydrogels as extracellular matrices for skeletal tissue engineering: state-of-the-art and novel application in organ printing. Tissue Eng 2007;13:1905–25.
[44] Fedorovich NE, Dewijn JR, Verbout AJ, Alblas J, Dhert WJA. Three-dimensional fiber deposition of cell-laden, viable, patterned constructs for bone tissue printing. Tissue Eng Pt A 2008;14:127–33.
[45] Fragonas E, Valente M, Pozzi-Mucelli M, Toffanin R, Rizzo R, Silvestri F, et al. Articular cartilage repair in rabbits by using suspensions of allogenic chondrocytes in alginate. Biomaterials 2000;21:795–801.
[46] Hauselmann HJ, Fernandes RJ, Mok SS, Schmid TM, Block JA, Aydelotte MB, et al. Phenotypic stability of bovine articular chondrocytes after long-term culture in alginate beads. J Cell Sci 1994;107:17–27.
[47] Hoemann CD, Sun J, Legare A, McKee MD, Buschmann MD. Tissue engineering of cartilage using an injectable and adhesive chitosan-based cell-delivery vehicle. Osteoarthr Cartilage 2005;13:318–29.
[48] Hosseinkhani H, Hosseinkhani M, Tian F, Kobayashi H, Tabata Y. Osteogenic differentiation of mesenchymal stem cells in self-assembled peptide-amphiphile nanofibers. Biomaterials 2006;27:4079–86.
[49] Iwashina T, Mochida J, Miyazaki T, Watanabe T, Iwabuchi S, Ando K, et al. Low-intensity pulsed ultrasound stimulates cell proliferation and proteoglycan production in rabbit intervertebral disc cells cultured in alginate. Biomaterials 2006;27:354–61.
[50] Kisiday J, Jin M, Kurz B, Hung H, Semino C, Zhang S, et al. Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division: implications for cartilage tissue repair. P Natl Acad Sci USA 2002;99:9996–10001.
[51] Klein TJ, Rizzi SC, Reichert JC, Georgi N, Malda J, Schuurman W, et al. Strategies for zonal cartilage repair using hydrogels. Macromol Biosci 2009;9:1049–58.
[52] Passaretti D, Silverman RP, Huang W, Kirchhoff CH, Ashiku S, Randolph MA, et al. Cultured chondrocytes produce injectable tissue-engineered cartilage in hydrogel polymer. Tissue Eng 2001;7:805–15.
[53] Peretti GM, Xu JW, Bonassar LJ, Kirchhoff CH, Yaremchuk MJ, Randolph MA. Review of injectable cartilage engineering using fibrin gel in mice and swine models. Tissue Eng 2006;12:1151–68.
[54] Roughley P, Hoemann C, DesRosiers E, Mwale F, Antoniou J, Alini M. The potential of chitosan-based gels containing intervertebral disc cells for nucleus pulposus supplementation. Biomaterials 2006;27:388–96.
[55] Stern S, Lindenhayn K, Schultz O, Perka C. Cultivation of porcine cells from the nucleus pulposus in a fibrin/hyaluronic acid matrix. Acta Orthop Scand 2000;71:496–502.
[56] Trivedi N, Keegan M, Steil GM, Hollister-Lock J, Hasenkamp WM, Colton CK, et al. Islets in alginate macrobeads reverse diabetes despite minimal acute insulin secretory responses. Transplantation 2001;71:203–11.
[57] Wong M, Siegrist M, Gaschen V, Park Y, Graber W, Studer D. Collagen fibrillogenesis by chondrocytes in alginate. Tissue Eng 2002;8:979–87.
[58] Bettinger CJ, Weinberg EJ, Kulig KM, Vacanti JP, Wang YD, Borenstein JT, et al. Three-dimensional microfluidic tissue-engineering scaffolds using a flexible biodegradable polymer. Adv Mater 2006;18:165–9.
[59] Anseth KS, Metters AT, Bryant SJ, Martens PJ, Elisseeff JH, Bowman CN. In situ forming degradable networks and their application in tissue engineering and drug delivery. J Control Release 2002;78:199–209.
[60] Bryant SJ, Cuy JL, Hauch KD, Ratner BD. Photo-patterning of porous hydrogels for tissue engineering. Biomaterials 2007;28:2978–86.
[61] Causa F, Netti PA, Ambrosio L. A multi-functional scaffold for tissue regeneration: the need to engineer a tissue analogue. Biomaterials 2007;28:5093–9.
[62] Chang CH, Liu HC, Lin CC, Chou CH, Lin FH. Gelatin-chondroitin-hyaluronan tri-copolymer scaffold for cartilage tissue engineering. Biomaterials 2003;24: 4853–8.
[63] Dang JM, Sun DDN, Shin-Ya Y, Sieber AN, Kostuik JP, Leong KW. Temperature-responsive hydroxybutyl chitosan for the culture of mesenchymal stem cells and intervertebral disk cells. Biomaterials 2006;27:406–18.
[64] Lawson MA, Barralet JE, Wang L, Shelton RM, Triffitt JT. Adhesion and growth of bone marrow stromal cells on modified alginate hydrogels. Tissue Eng 2004;10:1480–91.
[65] Lee J, Cuddihy MJ, Kotov NA. Three-dimensional cell culture matrices: state of the art. Tissue Eng Pt B-Rev 2008;14:61–86.
[66] Lee KY, Mooney DJ. Hydrogels for tissue engineering. Chem Rev 2001;101: 1869–79.
[67] Lutolf MR, Weber FE, Schmoekel HG, Schense JC, Kohler T, Muller R, et al. Repair of bone defects using synthetic mimetics of collagenous extracellular matrices. Nat Biotechnol 2003;21:513–8.
[68] Molinaro G, Leroux JC, Damas J, Adam A. Biocompatibility of thermosensitive chitosan-based hydrogels: an in vivo experimental approach to injectable biomaterials. Biomaterials 2002;23:2717–22.
[69] Nuttelman CR, Henry SM, Anseth KS. Synthesis and characterization of photocrosslinkable, degradable poly(vinyl alcohol)-based tissue engineering scaffolds. Biomaterials 2002;23:3617–26.
[70] Ponticiello MS, Schinagl RM, Kadiyala S, Barry FP. Gelatin-based resorbable sponge as a carrier matrix for human mesenchymal stem cells in cartilage regeneration therapy. J Biomed Mater Res 2000;52:246–55.
[71] Schuster M, Turecek C, Weigel G, Saf R, Stampfl J, Varga F, et al. Gelatin-based photopolymers for bone replacement materials. J Polym Sci Part a-Polymer Chem 2009;47:7078–89.
[72] Solchaga LA, Gao JZ, Dennis JE, Awadallah A, Lundberg M, Caplan AI, et al. Treatment of osteochondral defects with autologous bone marrow in a hyaluronan-based delivery vehicle. Tissue Eng 2002;8:333–47.
[73] Thebaud NB, Pierron D, Bareille R, Le Visage C, Letourneur D, Bordenave L. Human endothelial progenitor cell attachment to polysaccharide-based hydrogels: a pre-requisite for vascular tissue engineering. J Mater Science-Materials Med 2007;18:339–45.
[74] Tsang VL, Bhatia SN. Three-dimensional tissue fabrication. Adv Drug Del Rev 2004;56:1635–47.
[75] Wang L, Shelton RM, Cooper PR, Lawson M, Triffitt JT, Barralet JE. Evaluation of sodium alginate for bone marrow cell tissue engineering. Biomaterials 2003;24:3475–81.
[76] Weinand C, Pomerantseva I, Neville CM, Gupta R, Weinberg E, Madisch I, et al. Hydrogel-beta-tcp scaffolds and stem cells for tissue engineering bone. Bone 2006;38:555–63.
[77] Ahmed TAE, Dare EV, Hincke M. Fibrin: a versatile scaffold for tissue engineering applications. Tissue Eng Pt B-Rev 2008;14:199–215.
[78] Almany L, Seliktar D. Biosynthetic hydrogel scaffolds made from fibrinogen and polyethylene glycol for 3d cell cultures. Biomaterials 2005;26:2467–77.
[79] Chung HJ, Park TG. Self-assembled and nanostructured hydrogels for drug delivery and tissue engineering. Nano Today 2009;4:429–37.
[80] Di Martino A, Sittinger M, Risbud MV. Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. Biomaterials 2005;26:5983–90.
[81] Farrell E, O'Brien FJ, Doyle P, Fischer J, Yannas I, Harley BA, et al. A collagen-glycosaminoglycan scaffold supports adult rat mesenchymal stem cell differentiation along osteogenic and chondrogenic routes. Tissue Eng 2006; 12:459–68.
[82] Tibbitt MW, Anseth KS. Hydrogels as extracellular matrix mimics for 3d cell culture. Biotechnol Bioeng 2009;103:655–63.
[83] Wang XH, Yan YN, Zhang RJ. Recent trends and challenges in complex organ manufacturing. Tissue Eng Pt B-Rev 2010;16:189–97.
[84] Shin H, Temenoff JS, Bowden GC, Zygourakis K, Farach-Carson MC, Yaszemski MJ, et al. Osteogenic differentiation of rat bone marrow stromal cells cultured on arg-gly-asp modified hydrogels without dexamethasone and beta-glycerol phosphate. Biomaterials 2005;26:3645–54.
[85] Moller S, Weisser J, Bischoff S, Schnabelrauch M. Dextran and hyaluronan methacrylate based hydrogels as matrices for soft tissue reconstruction. Biomol Eng 2007;24:496–504.
[86] Shin H, Ruhe PQ, Mikos AG, Jansen JA. In vivo bone and soft tissue response to injectable, biodegradable oligo(poly(ethylene glycol) fumarate) hydrogels. Biomaterials 2003;24:3201–11.
[87] Wang TW, Spector M. Development of hyaluronic acid-based scaffolds for brain tissue engineering. Acta Biomater 2009;5:2371–84.
[88] Seo SJ, Kim IY, Choi YJ, Akaike T, Cho CS. Enhanced liver functions of hepatocytes cocultured with nih 3t3 in the alginate/galactosylated chitosan scaffold. Biomaterials 2006;27:1487–95.
[89] Mann BK, Gobin AS, Tsai AT, Schmedlen RH, West JL. Smooth muscle cell growth in photopolymerized hydrogels with cell adhesive and proteolytically degradable domains: synthetic ecm analogs for tissue engineering. Biomaterials 2001;22:3045–51.
[90] Erickson GR, Gimble JM, Franklin DM, Rice HE, Awad H, Guilak F. Chondrogenic potential of adipose tissue-derived stromal cells in vitro and in vivo. Biochem Bioph Res Co 2002;290:763–9.
[91] Cortiella J, Nichols JE, Kojima K, Bonassar LJ, Dargon P, Roy AK, et al. Tissue-engineered lung: an in vivo and in vitro comparison of polyglycolic acid and pluronic f-127 hydrogel/somatic lung progenitor cell constructs to support tissue growth. Tissue Eng 2006;12:1213–25.
[92] LaNasa SM, Bryant SJ. Influence of ecm proteins and their analogs on cells cultured on 2-d hydrogels for cardiac muscle tissue engineering. Acta Biomater 2009;5:2929–38.
[93] Van Vlierberghe S, Dubruel P, Lippens E, Masschaele B, Van Hoorebeke L, Cornelissen M, et al. Toward modulating the architecture of hydrogel scaffolds: curtains versus channels. J Mater Sci-Mater M 2008;19:1459–66.
[94] Fan JY, Shang Y, Yuan YJ, Yang J. Preparation and characterization of chitosan/galactosylated hyaluronic acid scaffolds for primary hepatocytes culture. J Mater Sci-Mater M 2010;21:319–27.
[95] Landers R, Pfister A, Hubner U, John H, Schmelzeisen R, Mulhaupt R. Fabrication of soft tissue engineering scaffolds by means of rapid prototyping techniques. J Mater Sci 2002;37:3107–16.
[96] Madaghiele M, Piccinno A, Saponaro M, Maffezzoli A, Sannino A. Collagen- and gelatine-based films sealing vascular prostheses: evaluation of the degree of crosslinking for optimal blood impermeability. J Mater Sci-Mater M 2009;20:1979–89.

[97] Noth U, Schupp K, Heymer A, Kall S, Jakob F, Schutze N, et al. Anterior cruciate ligament constructs fabricated from human mesenchymal stem cells in a collagen type i hydrogel. Cytotherapy 2005;7:447–55.
[98] Schmeichel KL, Bissell MJ. Modeling tissue-specific signaling and organ function in three dimensions. J Cell Sci 2003;116:2377–88.
[99] Dutta RC, Dutta AK. Cell-interactive 3d-scaffold; advances and applications. Biotechnol Adv 2009;27:334–9.
[100] Kirkpatrick CJ, Fuchs S, Hermanns MI, Peters K, Unger RE. Cell culture models of higher complexity in tissue engineering and regenerative medicine. Biomaterials 2007;28:5193–8.
[101] Hutmacher DW, Cool S. Concepts of scaffold-based tissue engineering-the rationale to use solid free-form fabrication techniques. J Cell Mol Med 2007;11:654–69.
[102] Mironov V, Boland T, Trusk T, Forgacs G, Markwald RR. Organ printing: computer-aided jet-based 3d tissue engineering. Trends Biotechnol 2003;21:157–61.
[103] Roach P, Eglin D, Rohde K, Perry CC. Modern biomaterials: a review-bulk properties and implications of surface modifications. J Mater Sci-Mater M 2007;18:1263–77.
[104] Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. Semin Immunol 2008;20:86–100.
[105] Azuma K, Nagaoka M, Cho CS, Akaike T. An artificial extracellular matrix created by hepatocyte growth factor fused to igg-fc. Biomaterials 2010;31:802–9.
[106] Hubbell JA. Bioactive biomaterials. Curr Opin Biotech 1999;10:123–9.
[107] Kikkawa Y, Takahashi N, Matsuda Y, Miwa T, Akizuki T, Kataoka A, et al. The influence of synthetic peptides derived from the laminin a1 chain on hepatocyte adhesion and gene expression. Biomaterials 2009;30:6888–95.
[108] Woo JH, Kim DY, Jo SY, Kang H, Noh I. Modification of the bulk properties of the porous poly(lactide-co-glycolide) scaffold by irradiation with a cyclotron ion beam with high energy for its application in tissue engineering. Biomed Mater 2009;4:044101.
[109] Zhu YB, Gao CY, Liu XY, Shen JC. Surface modification of polycaprolactone membrane via aminolysis and biomacromolecule immobilization for promoting cytocompatibility of human endothelial cells. Biomacromolecules 2002;3:1312–9.
[110] Zhu YB, Gao CY, Shen JC. Surface modification of polycaprolactone with poly(methacrylic acid) and gelatin covalent immobilization for promoting its cytocompatibility. Biomaterials 2002;23:4889–95.
[111] Chu PK, Chen JY, Wang LP, Huang N. Plasma-surface modification of biomaterials. Mat Sci Eng R 2002;36:143–206.
[112] Desmet T, Morent R, De Geyter N, Leys C, Schacht E, Dubruel P. Nonthermal plasma technology as a versatile strategy for polymeric biomaterials surface modification: a review. Biomacromolecules 2009;10:2351–78.
[113] Tsioptsias C, Tsivintzelis I, Papadopoulou L, Pallayiotou C. A novel method for producing tissue engineering scaffolds from chitin, chitin-hydroxyapatite, and cellulose. Mat Sci Eng C-Bio S 2009;29:159–64.
[114] Martin L, Alonso M, Girotti A, Arias FJ, Rodriguez-Cabello JC. Synthesis and characterization of macroporous thermosensitive hydrogels from recombinant elastin-like polymers. Biomacromolecules 2009;10:3015–22.
[115] Pathi SP, Kowalczewski C, Tadipatri R, Fischbach C. A novel 3-d mineralized tumor model to study breast cancer bone metastasis. PloS One 2010;5:e8849.
[116] Zhu XH, Arifin DY, Khoo BH, Hua JS, Wang CH. Study of cell seeding on porous poly(d, l-lactic-co-glycolic acid) sponge and growth in a couette-taylor bioreactor. Chem Eng Sci 2010;65:2108–17.
[117] Salerno A, Netti PA, Di Maio E, Iannace S. Engineering of foamed structures for biomedical application. J Cell Plast 2009;45:103–17.
[118] Gomes ME, Azevedo HS, Moreira AR, Ella V, Kellomaki M, Reis RL. Starch-poly(epsilon-caprolactone) and starch-poly(lactic acid) fibre-mesh scaffolds for bone tissue engineering applications: structure, mechanical properties and degradation behaviour. J Tissue Eng Regen M 2008;2:243–52.
[119] Mooney DT, Mazzoni CL, Breuer C, McNamara K, Hern D, Vacanti JP, et al. Stabilized polyglycolic acid fibre based tubes for tissue engineering. Biomaterials 1996;17:115–24.
[120] Liu XH, Ma PX. Phase separation, pore structure, and properties of nanofibrous gelatin scaffolds. Biomaterials 2009;30:4094–103.
[121] Nichols MD, Scott EA, Elbert DL. Factors affecting size and swelling of poly(ethylene glycol) microspheres formed in aqueous sodium sulfate solutions without surfactants. Biomaterials 2009;30:5283–91.
[122] Lee S, Seong SC, Lee JH, Han IK, Oh SH, Cho KJ, et al. Porous polymer prosthesis for meniscal regeneration. Key Eng Mat 2007;342–343:33–6.
[123] Wang YY, Liu L, Guo SR. Characterization of biodegradable and cytocompatible nano-hydroxyapatite/polycaprolactone porous scaffolds in degradation in vitro. Polym Degrad Stabil 2010;95:207–13.
[124] Mu YH, Li YB, Wang MB, Xu FL, Zhang X, Tian ZY. Novel method to fabricate porous n-ha/pva hydrogel scaffolds. Mater Sci Forum 2006;510–511:878–81.
[125] Sinha A, Guha A. Biomimetic patterning of polymer hydrogels with hydroxyapatite nanoparticles. Mat Sci Eng C Bio S 2009;29:1330–3.
[126] Mironov V, Kasyanov V, Shu XZ, Eisenberg C, Eisenberg L, Gonda S, et al. Fabrication of tubular tissue constructs by centrifugal casting of cells suspended in an in situ crosslinkable hyaluronan-gelatin hydrogel. Biomaterials 2005;26:7628–35.
[127] Pitarresi G, Palumbo FS, Calabrese R, Craparo EF, Giammona G. Crosslinked hyaluronan with a protein-like polymer: novel bioresorbable films for biomedical applications. J Biomed Mater Res A 2008;84A:413–24.
[128] Estelles JM, Vidaurre A, Duenas JMM, Cortazar IC. Physical characterization of polycaprolactone scaffolds. J Mater Sci Mater M 2008;19:189–95.
[129] Yang SF, Leong KF, Du ZH, Chua CK. The design of scaffolds for use in tissue engineering. Part I. Traditional factors. Tissue Eng 2001;7:679–89.
[130] Chua CK, Leong KF, Lim CS. Rapid prototyping: principles and applications. Singapore: World Scientific Publishing; 2004.
[131] Yang SF, Leong KF, Du ZH, Chua CK. The design of scaffolds for use in tissue engineering. Part II. Rapid prototyping techniques. Tissue Eng 2002;8:1–11.
[132] Melchels FPW, Feijen J, Grijpma DW. A review on stereolithography and its applications in biomedical engineering. Biomaterials 2010;31:6121–30.
[133] Arcaute K, Mann BK, Wicker RB. Stereolithography of three-dimensional bioactive poly(ethylene glycol) constructs with encapsulated cells. Ann Biomed Eng 2006;34:1429–41.
[134] Liska R, Schuster M, Infuhr R, Tureeek C, Fritscher C, Seidl B, et al. Photopolymers for rapid prototyping. J Coat Technol Res 2007;4:505–10.
[135] Lee SJ, Rhie JW, Cho DW. Development of three-dimensional alginate encapsulated chondrocyte hybrid scaffold using microstereolithography. J Manuf Sci E-T ASME 2008;130:021007.
[136] Lee SJ, Kang T, Rhie JW, Cho DW. Development of three-dimensional hybrid scaffold using chondrocyte-encapsulated alginate hydrogel. Sensor Mater 2007;19:445–51.
[137] Lee SJ, Kang HW, Park JK, Rhie JW, Hahn SK, Cho DW. Application of microstereolithography in the development of three-dimensional cartilage regeneration scaffolds. Biomed Microdevices 2008;10:233–41.
[138] Dhariwala B, Hunt E, Boland T. Rapid prototyping of tissue-engineering constructs, using photopolymerizable hydrogels and stereolithography. Tissue Eng 2004;10:1316–22.
[139] Hutmacher DW, Sittinger M, Risbud MV. Scaffold-based tissue engineering: rationale for computer-aided design and solid free-form fabrication systems. Trends Biotechnol 2004;22:354–62.
[140] Lu Y, Mapili G, Suhali G, Chen SC, Roy K. A digital micro-mirror device-based system for the microfabrication of complex, spatially patterned tissue engineering scaffolds. J Biomed Mater Res A 2006;77A:396–405.
[141] Choi JW, Wicker R, Lee SH, Choi KH, Ha CS, Chung I. Fabrication of 3d biocompatible/biodegradable micro-scaffolds using dynamic mask projection microstereolithography. J Mater Process Tech 2009;209:5494–503.
[142] Han LH, Mapili G, Chen S, Roy K. Projection microfabrication of three-dimensional scaffolds for tissue engineering. J Manuf Sci E-T ASME 2008;130:021005.
[143] Itoga K, Yamato M, Kobayashi J, Kikuchi A, Okano T. Cell micropatterning using photopolymerization with a liquid crystal device commercial projector. Biomaterials 2004;25:2047–53.
[144] Sun C, Fang N, Wu DM, Zhang X. Projection micro-stereolithography using digital micro-mirror dynamic mask. Sensor Actuat A Phys 2005;121:113–20.
[145] Gu P, Zhang X, Zeng Y, Ferguson B. Quality analysis and optimization of solid ground curing process. J Manuf Syst 2001;20:250–63.
[146] Zhang X, Zhou B, Zeng Y, Gu P. Model layout optimization for solid ground curing rapid prototyping processes. Robot CIM Int Manuf 2002;18:41–51.
[147] Xing JF, Dong XZ, Chen WQ, Duan XM, Takeyasu N, Tanaka T, et al. Improving spatial resolution of two-photon microfabrication by using photoinitiator with high initiating efficiency. Appl Phys Lett 2007;90:131106.
[148] Yu T, Chiellini F, Schmaljohan D, Solaro R, Ober C. Microfabrication of hydrogels for biomedical applications. In: Fedynyshyn TH, editor. Advances in resist technology and processing. Washington: Bellingham; 2002. p. 854–60.
[149] Mapili G, Lu Y, Chen SC, Roy K. Laser-layered microfabrication of spatially patterned functionalized tissue-engineering scaffolds. J Biomed Mater Res B 2005;75B:414–24.
[150] Arcaute K, Mann B, Wicker R. Stereolithography of spatially controlled multi-material bioactive poly(ethylene glycol) scaffolds. Acta Biomater 2010;6:1047–54.
[151] ASME Arcaute K, Ochoa L, Mann BK, Wicker RB. Stereolithography of PEG hydrogel multi-lumen nerve regeneration conduits. Manuf Eng Div Asme; 2005:161–7.
[152] Arcaute K, Ochoa L, Medina F, Elkins C, Mann B, Wicker R. Three-dimensional PEG hydrogel construct fabrication using stereolithography. Mater Res Soc Symp p. 191–197.
[153] Yasar O, Lan S, Starly B. A lindenmayer system-based approach for the design of nutrient delivery networks in tissue constructs. Biofabrication 2009;1:045004.
[154] Koh WG, Revzin A, Pishko MV. Poly(ethylene glycol) hydrogel microstructures encapsulating living cells. Langmuir 2002;18:2459–62.
[155] Khademhosseini A, Eng G, Yeh J, Fukuda J, Blumling J, Langer R, et al. Micromolding of photocrosslinkable hyaluronic acid for cell encapsulation and entrapment. J Biomed Mater Res A 2006;79A:522–32.
[156] Luo Y, Shoichet MS. A photolabile hydrogel for guided three-dimensional cell growth and migration. Nat Mat 2004;3:249–53.
[157] Khademhosseini A, Langer R. Microengineered hydrogels for tissue engineering. Biomaterials 2007;28:5087–92.
[158] Barry RA, Shepherd RF, Hanson JN, Nuzzo RG, Wiltzius P, Lewis JA. Direct-write assembly of 3D hydrogel scaffolds for guided cell growth. Adv Mater 2009;21:2407–10.

[159] Yuan D, Lasagni As, Shao P, Das S. Rapid prototyping of microstructured hydrogels via laser direct-write and laser interference photopolymerisation. Virtual Phys Prototyping 2008;3:221–9.
[160] Schade R, Weiss T, Berg A, Schnabelrauch M, Liefeith K. Two-photon techniques in tissue engineering. Int J Artif Organs 2010;33:219–27.
[161] Ovsianikov A, Gruene M, Pflaum M, Koch L, Maiorana F, Wilhelmi M, et al. Laser printing of cells into 3D scaffolds. Biofabrication 2010;2:014104.
[162] Van den Bulcke A, Bogdanov B, De Rooze N, Schacht E, Cornelissen M, Berghmans H. Structural and rheological properties of methacrylamide modified gelatin hydrogels. Biomacromolecules 2000;1:31–8.
[163] Ovsianikov A, Deiwick A, Van Vlierberghe S, Pflaum M, Wilhelmi M, Dubruel P, et al. Laser fabrication of 3D gelatin scaffolds for the generation of bioartificial tissues. Materials 2011;4:288–99.
[164] Ovsianikov A, Deiwick A, Van Vlierberghe S, Dubruel P, Moller L, Drager G, et al. Laser fabrication of three-dimensional CAD scaffolds from photosensitive gelatin for applications in tissue engineering. Biomacromolecules 2011;12:851–8.
[165] Engelhardt S, Hoch E, Borchers K, Meyer W, Kruger H, Tovar GEM, et al. Fabrication of 2D protein microstructures and 3D polymer-protein hybrid microstructures by two-photon polymerization. Biofabrication 2011;3:025003.
[166] Khalil S, Nam J, Sun W. Multi-nozzle deposition for construction of 3D biopolymer tissue scaffolds. Rapid Prototyping J 2005;11:9–17.
[167] Liu L, Xiong Z, Yan YN, Zhang RJ, Wang XH, Jin L. Multinozzle low-temperature deposition system for construction of gradient tissue engineering scaffolds. J Biomed Mater Res B 2009;88B:254–63.
[168] Vozzi G, Ahluwalia A. Microfabrication for tissue engineering: rethinking the cells-on-a scaffold approach. J Mater Chem 2007;17:1248–54.
[169] Mariani M, Rosatini F, Vozzi G, Previti A, Ahluwalia A. Characterization of tissue-engineered scaffolds microfabricated with pam. Tissue Eng 2006;12:547–57.
[170] Vozzi G, Previti A, De Rossi D, Ahluwalia A. Microsyringe-based deposition of two-dimensional and three-dimensional polymer scaffolds with a well-defined geometry for application to tissue engineering. Tissue Eng 2002;8:1089–98.
[171] Vozzi G, Previti A, Ciaravella G, Ahluwalia A. Microfabricated fractal branching networks. J Biomed Mater Res A 2004;71A:326–33.
[172] Tirella A, Vozzi G, Ahluwalia A. Biomimicry of pam microfabricated hydrogel scaffold. Nip24/Digital fabrication 2008: 24th international conference on digital printing technologies. Tech Program Proc; 2008:496–500.
[173] Tirella A, Orsini A, Vozzi G, Ahluwalia A. A phase diagram for microfabrication of geometrically controlled hydrogel scaffolds. Biofabrication 2009;1:045002.
[174] Xiong Z, Yan YN, Wang SG, Zhang RJ, Zhang C. Fabrication of porous scaffolds for bone tissue engineering via low-temperature deposition. Scripta Mater 2002;46:771–6.
[175] Xu W, Wang XH, Yan YN, Zhang RJ. Rapid protoyping of polyurethane for the creation of vascular systems. J Bioact Compat Pol 2008;23:103–15.
[176] Liu L, Xiong Z, Zhang RJ, Jin L, Yan YN. A novel osteochondral scaffold fabricated via multi-nozzle low-temperature deposition manufacturing. J Bioact Compat Pol 2009;24:18–30.
[177] Khalil S, Nam J, Sun W. Biopolymer deposition for freeform fabrication of tissue engineered scaffolds. Proc IEEE 30th Annu Northeast Bioeng Conf; 2004:136–7.
[178] Zhuo X, Yongnian Y, Shenguo W, Renji Z, Chao Z. Fabrication of porous scaffolds for bone tissue engineering via low-temperature deposition. Scripta Mater 2002;46:771–6.
[179] Landers R, Mulhaupt R. Desktop manufacturing of complex objects, prototypes and biomedical scaffolds by means of computer-assisted design combined with computer-guided 3D plotting of polymers and reactive oligomers. Macromol Mater Eng 2000;282:17–21.
[180] Kim GH, Son JG. 3D polycarprolactone (pcl) scaffold with hierarchical structure fabricated by a piezoelectric transducer (pzt)-assisted bioplotter. Appl Phys A Mater 2009;94:781–5.
[181] Ang TH, Sultana FSA, Hutmacher DW, Wong YS, Fuh JYH, Mo XM, et al. Fabrication of 3D chitosan-hydroxyapatite scaffolds using a robotic dispensing system. Mat Sci Eng C Bio S 2002;20:35–42.
[182] Cesarano J. A review of robocasting technology. Mater Res Soc Symp P 1999; 542:133–9.
[183] Franco J, Hunger P, Launey ME, Tomsia AP, Saiz E. Direct write assembly of calcium phosphate scaffolds using a water-based hydrogel. Acta Biomater 2010;6:218–28.
[184] Munch E, Franco J, Deville S, Hunger P, Saiz E, Tomsia AP. Porous ceramic scaffolds with complex architectures. Jom-US 2008;60:54–8.
[185] Smay JE, Gratson GM, Shepherd RF, Cesarano J, Lewis JA. Directed colloidal assembly of 3D periodic structures. Adv Mater 2002;14:1279–83.
[186] Gratson GM, Xu MJ, Lewis JA. Microperiodic structures-direct writing of three-dimensional webs. Nature 2004;428:386.
[187] Duoss EB, Twardowski M, Lewis JA. Sol-gel inks for direct-write assembly of functional oxides. Adv Mater 2007;19:3485–9.
[188] van Osch THJ, Perelaer J, de Laat AWM, Schubert US. Inkjet printing of narrow conductive tracks on untreated polymeric substrates. Adv Mater 2008;20:343–5.
[189] Guo JJ, Lewis JA. Aggregation effects on the compressive flow properties and drying behavior of colloidal silica suspensions. J Am Ceram Soc 1999;82: 2345–58.
[190] Iwami K, Noda T, Ishida K, Morishima K, Nakamura M, Umeda N. Bio rapid prototyping by extruding/aspirating/refilling thermoreversible hydrogel. Biofabrication 2010;2:014108.
[191] Li SJ, Xiong Z, Wang XH, Yan YN, Liu HX, Zhang RJ. Direct fabrication of a hybrid cell/hydrogel construct by a double-nozzle assembling technology. J Bioact Compat Pol 2009;24:249–65.
[192] Li SJ, Yan YN, Xiong Z, Weng CY, Zhang RJ, Wang XH. Gradient hydrogel construct based on an improved cell assembling system. J Bioact Compat Pol 2009;24:84–99.
[193] Smith CM, Stone AL, Parkhill RL, Stewart RL, Simpkins MW, Kachurin AM, et al. Three-dimensional bioassembly tool for generating viable tissue-engineered constructs. Tissue Eng 2004;10:1566–76.
[194] Lee W, Debasitis JC, Lee VK, Lee JH, Fischer K, Edminster K, et al. Multi-layered culture of human skin fibroblasts and keratinocytes through three-dimensional freeform fabrication. Biomaterials 2009;30: 1587–95.
[195] Cheng J, Lin F, Liu HX, Yan YN, Wang XH, Zhang R, et al. Rheological properties of cell-hydrogel composites extruding through small-diameter tips. J Manuf Sci E T ASME 2008;130:021014.
[196] Xu M, Wang X, Yan Y, Yao R, Ge Y. An cell-assembly derived physiological 3D model of the metabolic syndrome, based on adipose-derived stromal cells and a gelatin/alginate/fibrinogen matrix. Biomaterials 2010;31: 3868–77.
[197] Skardal A, Zhang J, McCoard L, Xu X, Oottamasathien S, Prestwich GD. Photocrosslinkable hyaluronan-gelatin hydrogels for two-step bioprinting. Tissue Eng Part A 2010;16:2675–85.
[198] Chang R, Sun W. Effects of dispensing pressure and nozzle diameter on cell survival from solid freeform fabrication-based direct cell writing. Tissue Eng Part A 2008;14:41–8.
[199] Wong JY, Velasco A, Rajagopalan P, Pham Q. Directed movement of vascular smooth muscle cells on gradient-compliant hydrogels. Langmuir 2003;19: 1908–13.
[200] Vozzi G, Flaim C, Ahluwalia A, Bhatia S. Fabrication of plga scaffolds using soft lithography and microsyringe deposition. Biomaterials 2003;24: 2533–40.
[201] Cohen DL, Lipton JI, Bonassar LJ, Lipson H. Additive manufacturing for in situ repair of osteochondral defects. Biofabrication 2010;2:035004.
[202] Lixandrao AL, Noritomi PY, da Silva JVL, Neto PI, Cheung PYC, Colangelo N, et al. Construction and adaptation of an open source rapid prototyping machine for biomedical research purposes-a multinational collaborative development. Innovative Dev Des Manufacturing; 2010:469–73.
[203] Zhang T, Yan YN, Wang XH, Xiong Z, Lin F, Wu RD, et al. Three-dimensional gelatin and gelatin/hyaluronan hydrogel structures for traumatic brain injury. J Bioact Compat Pol 2007;22:19–29.
[204] Xu W, Wang XH, Yan YN, Zheng W, Xiong Z, Lin F, et al. Rapid prototyping three-dimensional cell/gelatin/fibrinogen constructs for medical regeneration. J Bioact Compat Pol 2007;22:363–77.
[205] Yan YN, Wang XH, Xiong Z, Liu HX, Liu F, Lin F, et al. Direct construction of a three-dimensional structure with cells and hydrogel. J Bioact Compat Pol 2005;20:259–69.
[206] Yan YN, Wang XH, Pan YQ, Liu HX, Cheng J, Xiong Z, et al. Fabrication of viable tissue-engineered constructs with 3D cell-assembly technique. Biomaterials 2005;26:5864–71.
[207] Rucker M, Laschke MW, Junker D, Carvalho C, Schramm A, Mulhaupt R, et al. Angiogenic and inflammatory response to biodegradable scaffolds in dorsal skinfold chambers of mice. Biomaterials 2006;27:5027–38.
[208] Wang XH, Yan YN, Pan YQ, Xiong Z, Liu HX, Cheng B, et al. Generation of three-dimensional hepatocyte/gelatin structures with rapid prototyping system. Tissue Eng 2006;12:83–90.
[209] Schuurman W, Khristov V, Pot MW, van Weeren PR, Dhert WJA, Malda J. Bioprinting of hybrid tissue constructs with tailorable mechanical properties. Biofabrication 2011;3:021001.
[210] Shim JH, Kim JY, Park M, Park J, Cho DW. Development of a hybrid scaffold with synthetic biomaterials and hydrogel using solid freeform fabrication technology. Biofabrication 2011;3:034102.
[211] Landers R, Hubner U, Schmelzeisen R, Mulhaupt R. Rapid prototyping of scaffolds derived from thermoreversible hydrogels and tailored for applications in tissue engineering. Biomaterials 2002;23:4437–47.
[212] Maher PS, Keatch RP, Donnelly K, Mackay RE, Paxton JZ. Construction of 3D biological matrices using rapid prototyping technology. Rapid Prototyping J 2009;15:204–10.
[213] Fedorovich NE, Swennen I, Girones J, Moroni L, van Blitterswijk CA, Schacht E, et al. Evaluation of photocrosslinked lutrol hydrogel for tissue printing applications. Biomacromolecules 2009;10:1689–96.
[214] Snyder JE, Hamid Q, Wang C, Chang R, Emami K, Wu H, et al. Bioprinting cell-laden matrigel for radioprotection study of liver by pro-drug conversion in a dual-tissue microfluidic chip. Biofabrication 2011;3:034112.
[215] Xu Wei, Yan Yongnian, Zhang Renji. Rapid protyping of polyurethane for the creation of vascular systems. J Bioact Compat Pol 2008;23:103–14.
[216] Xie BJ, Parkhill RL, Warren WL, Smay JE. Direct writing of three-dimensional polymer scaffolds using colloidal gels. Adv Funct Mater 2006;16:1685–93.
[217] Tartarisco G, Gallone G, Carpi F, Vozzi G. Polyurethane unimorph bender microfabricated with pressure assisted microsyringe (pam) for biomedical applications. Mat Sci Eng C Bio S 2009;29:1835–41.
[218] Sachs E, Cima M, Cornie J. Three-dimensional printing: rapid tooling and prototypes directly from a cad model. CIRP Ann Manufacturing Technol 1990;39:201–4.

[219] Nakamura M, Kobayashi A, Takagi F, Watanabe A, Hiruma Y, Ohuchi K, et al. Biocompatible inkjet printing technique for designed seeding of individual living cells. Tissue Eng 2005;11:1658–66.
[220] Cima M, Sachs E, Fan TL, Bredt JF, Michaels SP, Khanuja S, et al. Three-dimensional printing techniques; 1993. US Patent No 5204055.
[221] Pfister A, Landers R, Laib A, Hubner U, Schmelzeisen R, Mulhaupt R. Biofunctional rapid prototyping for tissue-engineering applications: 3D bioplotting versus 3D printing. J Polym Sci A 2004;42:624–38.
[222] Lam CXF, Mo XM, Teoh SH, Hutmacher DW. Scaffold development using 3D printing with a starch-based polymer. Mat Sci Eng C Bio S 2002;20:49–56.
[223] Utela BR, Storti D, Anderson RL, Ganter M. A review of process development steps for new material systems in three dimensional printing (3DP). J Manufacturing Process 2008;10:96–104.
[224] Boland T, Tao X, Damon BJ, Manley B, Kesari P, Jalota S, et al. Drop-on-demand printing of cells and materials for designer tissue constructs. Mat Sci Eng C Bio S 2007;27:372–6.
[225] Sun J, Ng JH, Fuh YH, Wong YS, Loh HT, Xu Q. Comparison of micro-dispensing performance between micro-valve and piezoelectric printhead. Microsyst Technol 2009;15:1437–48.
[226] Wu BM, Borland SW, Giordano RA, Cima LG, Sachs EM, Cima MJ. Solid free-form fabrication of drug delivery devices. J Control Release 1996;40:77–87.
[227] Sanjana NE, Fuller SB. A fast flexible ink-jet printing method for patterning dissociated neurons in culture. J Neurosci Meth 2004;136:151–63.
[228] Xu T, Gregory CA, Molnar P, Cui X, Jalota S, Bhaduri SB, et al. Viability and electrophysiology of neural cell structures generated by the inkjet printing method. Biomaterials 2006;27:3580–8.
[229] Koegler WS, Griffith LG. Osteoblast response to plga tissue engineering scaffolds with peo modified surface chemistries and demonstration of patterned cell response. Biomaterials 2004;25:2819–30.
[230] Cui XF, Boland T. Human microvasculature fabrication using thermal inkjet printing technology. Biomaterials 2009;30:6221–7.
[231] Sachlos E, Reis N, Ainsley C, Derby B, Czernuszka JT. Novel collagen scaffolds with predefined internal morphology made by solid freeform fabrication. Biomaterials 2003;24:1487–97.
[232] Yeong WY, Chua CK, Leong KF, Chandrasekaran M, Lee MW. Comparison of drying methods in the fabrication of collagen scaffold via indirect rapid prototyping. J Biomed Mater Res B 2007;82B:260–6.
[233] Sastry SV, Nyshadham JR, Fix JA. Recent technological advances in oral drug delivery - a review. Pharm Sci Technol 2000;3:138–45.
[234] Leong KF, Cheah CM, Chua CK. Solid freeform fabrication of three-dimensional scaffolds for engineering replacement tissues and organs. Biomaterials 2003;24:2363–78.
[235] Suwanprateeb J. Improvement in mechanical properties of three-dimensional printing parts made from natural polymers reinforced by acrylate resin for biomedical applications: a double infiltration approach. Polym Int 2006;55:57–62.
[236] Boland T, Mironov V, Gutowska A, Roth EA, Markwald RR. Cell and organ printing 2: fusion of cell aggregates in three-dimensional gels. Anat Rec Part A 2003;272A:497–502.
[237] Smay JE, Cesarano J, Lewis JA. Colloidal inks for directed assembly of 3-d periodic structures. Langmuir 2002;18:5429–37.
[238] Simon JL, Michna S, Lewis JA, Rekow ED, Thompson VP, Smay JE, et al. In vivo bone response to 3d periodic hydroxyapatite scaffolds assembled by direct ink writing. J Biomed Mater Res A 2007;83A:747–58.
[239] Geng L, Feng W, Hutmacher DW, Wong YS, Loh HT, Fuh JYH. Direct writing of chitosan scaffolds using a robotic system. Rapid Prototyping J 2005;11:90–7.
[240] Yan YN, Xiong Z, Hu YY, Wang SG, Zhang RJ, Zhang C. Layered manufacturing of tissue engineering scaffolds via multi-nozzle deposition. Mater Lett 2003;57:2623–8.
[241] Therriault D, White SR, Lewis JA. Chaotic mixing in three-dimensional microvascular networks fabricated by direct-write assembly. Nat Mater 2003;2:265–71.
[242] Hollister SJ. Porous scaffold design for tissue engineering. Nat Mater 2005;4:518–24.
[243] Rumi M, Ehrlich JE, Heikal AA, Perry JW, Barlow S, Hu ZY, et al. Structure-property relationships for two-photon absorbing chromophores: bis-donor diphenylpolyene and bis(styryl)benzene derivatives. J Am Chem Soc 2000;122:9500–10.
[244] Kuebler SM, Braun KL, Zhou WH, Cammack JK, Yu TY, Ober CK, et al. Design and application of high-sensitivity two-photon initiators for three-dimensional microfabrication. J Photoch Photobio A 2003;158:163–70.
[245] Cellesi F, Tirelli N, Hubbell JA. Towards a fully-synthetic substitute of alginate: development of a new process using thermal gelation and chemical cross-linking. Biomaterials 2004;25:5115–24.
[246] Niu GG, Zhang HB, Song L, Cui XP, Cao H, Zheng YD, et al. Thiol/acrylate-modified peo-ppo-peo triblocks used as reactive and thermosensitive copolymers. Biomacromolecules 2008;9:2621–8.
[247] Niu GG, Du FY, Song L, Zhang HB, Yang J, Cao H, et al. Synthesis and characterization of reactive poloxamer 407s for biomedical applications. J Control Release 2009;138:49–56.
[248] Shu XZ, Liu YC, Palumbo FS, Lu Y, Prestwich GD. In situ crosslinkable hyaluronan hydrogels for tissue engineering. Biomaterials 2004;25:1339–48.
[249] Du YJ, Brash JL. Synthesis and characterization of thiol-terminated poly(ethylene oxide) for chemisorption to gold surface. J Appl Polym Sci 2003;90:594–607.
[250] Brink KS, Yang PJ, Temenoff JS. Degradative properties and cytocompatibility of a mixed-mode hydrogel containing oligo poly(ethylene glycol) fumarate and poly(ethylene glycol)dithiol. Acta Biomater 2009;5:570–9.
[251] Aimetti AA, Machen AJ, Anseth KS. Poly(ethylene glycol) hydrogels formed by thiol-ene photopolymerization for enzyme-responsive protein delivery. Biomaterials 2009;30:6048–54.
[252] Govender S, Swart R. Surfactant formulations for multi-functional surface modification. Colloid Surf A 2008;331:97–102.
[253] Lee TY, Bowman CN. The effect of functionalized nanoparticles on thiol-ene polymerization kinetics. Polymer 2006;47:6057–65.
[254] Rydholm AE, Bowman CN, Anseth KS. Degradable thiol-acrylate photopolymers: polymerization and degradation behavior of an in situ forming biomaterial. Biomaterials 2005;26:4495–506.
[255] Lutolf MP, Raeber GP, Zisch AH, Tirelli N, Hubbell JA. Cell-responsive synthetic hydrogels. Adv Mater 2003;15:888–92.
[256] Liang HC, Chang WH, Liang HF, Lee MH, Sung HW. Crosslinking structures of gelatin hydrogels crosslinked with genipin or a water-soluble carbodiimide. J Appl Polym Sci 2004;91:4017–26.
[257] Bryant SJ, Nuttelman CR, Anseth KS. Cytocompatibility of uv and visible light photoinitiating systems on cultured nih/3t3 fibroblasts in vitro. J Biomat Sci-Polym Sci 2000;11:439–57.
[258] Williams CG, Malik AN, Kim TK, Manson PN, Elisseeff JH. Variable cytocompatibility of six cell lines with photoinitiators used for polymerizing hydrogels and cell encapsulation. Biomaterials 2005;26:1211–8.
[259] Kizilel S, Sawardecker E, Teymour F, Perez-Luna VH. Sequential formation of covalently bonded hydrogel multilayers through surface initiated photopolymerization. Biomaterials 2006;27:1209–15.
[260] Desai PN, Yuan Q, Yang H. Synthesis and characterization of photocurable polyamidoamine dendrimer hydrogels as a versatile platform for tissue engineering and drug delivery. Biomacromolecules 2010;11:666–73.
[261] Fedorovich NE, Oudshoorn MH, van Geemen D, Hennink WE, Alblas J, Dhert WJA. The effect of photopolymerization on stem cells embedded in hydrogels. Biomaterials 2009;30:344–53.
[262] Duan SF, Zhu W, Yu L, Ding JD. Negative cooperative effect of cytotoxicity of a di-component initiating system for a novel injectable tissue engineering hydrogel. Chin Sci Bull 2005;50:1093–6.
[263] Sarac AS. Redox polymerization. Prog Polym Sci 1999;24:1149–204.
[264] Jakab K, Norotte C, Marga F, Murphy K, Vunjak-Novakovic G, Forgacs G. Tissue engineering by self-assembly and bio-printing of living cells. Biofabrication 2010;2:022001.
[265] Langer R. Editorial: tissue engineering: perspectives, challenges, and future directions. Tissue Eng 2007;13:1–2.
[266] Mironov V, Visconti RP, Kasyanov V, Forgacs G, Drake CJ, Markwald RR. Organ printing: tissue spheroids as building blocks. Biomaterials 2009;30:2164–74.
[267] Whitesides GM, Boncheva M. Beyond molecules: self-assembly of mesoscopic and macroscopic components. P Natl Acad Sci USA 2002;99:4769–74.
[268] Whitesides GM, Grzybowski B. Self-assembly at all scales. Science 2002;295:2418–21.
[269] L'heureux N, Paquet S, Labbe R, Germain L, Auger FA. A completely biological tissue-engineered human blood vessel. FASEB J 1998;12:47–56.
[270] L'heureux N, Dusserre N, Konig G, Horgan M, Kyles A, Gregory CR, et al. First use of a completely biological human tissue engineered blood vessel in a primate model. Circulation 2004;110:508.
[271] Hannachi IE, Yamato M, Okano T. Cell sheet technology and cell patterning for biofabrication. Biofabrication 2009;1:022002.
[272] Haraguchi Y, Shimizu T, Yamato M, Kikuchi A, Okano T. Electrical coupling of cardiomyocyte sheets occurs rapidly via functional gap junction formation. Biomaterials 2006;27:4765–74.
[273] Shimizu T, Sekine H, Isoi Y, Yamato M, Kikuchi A, Okano T. Long-term survival and growth of pulsatile myocardial tissue grafts engineered by the layering of cardiomyocyte sheets. Tissue Eng 2006;12:499–507.
[274] Nishida K, Yamato M, Hayashida Y, Watanabe K, Yamamoto K, Adachi E, et al. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. New Engl J Med 2004;351:1187–96.
[275] Barron JA, Wu P, Ladouceur HD, Ringeisen BR. Biological laser printing: a novel technique for creating heterogeneous 3-dimensional cell patterns. Biomed Microdevices 2004;6:139–47.
[276] Guillemot F, Souquet A, Catros S, Guillotin B, Lopez J, Faucon M, et al. High-throughput laser printing of cells and biomaterials for tissue engineering. Acta Biomater 2010;6:2494–500.
[277] Guillotin B, Souquet A, Catros S, Duocastella M, Pippenger B, Bellance S, et al. Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. Biomaterials 2010;31:7250–6.
[278] Wang W, Huang Y, Grujicic M, Chrisey DB. Study of impact-induced mechanical effects in cell direct writing using smooth particle hydrodynamic method. J Manuf Sci E T ASME 2008;130:021012.

[279] Boland T, Xu T, Damon B, Cui X. Application of inkjet printing to tissue engineering. Biotechnol J 2006;1:910–7.
[280] Wilson WC, Boland T. Cell and organ printing 1: protein and cell printers. Anat Rec Part A 2003;272A:491–6.
[281] Campbell PG, Weiss LE. Tissue engineering with the aid of inkjet printers. Expert Opin Biol Th 2007;7:1123–7.
[282] Xu T, Jin J, Gregory C, Hickman JJ, Boland T. Inkjet printing of viable mammalian cells. Biomaterials 2005;26:93–9.
[283] Yamazoe H, Tanabe T. Cell micropatterning on an albumin-based substrate using an inkjet printing technique. J Biomed Mater Res A 2009;91A:1202–9.
[284] Calvert P. Printing cells. Science 2007;318:208–9.
[285] Norotte C, Marga FS, Niklason LE, Forgacs G. Scaffold-free vascular tissue engineering using bioprinting. Biomaterials 2009;30:5910–7.
[286] Skardal A, Zhang JX, Prestwich GD. Bioprinting vessel-like constructs using hyaluronan hydrogels crosslinked with tetrahedral polyethylene glycol tetracrylates. Biomaterials 2010;31:6173–81.
[287] Lee YB, Polio S, Lee W, Dai GH, Menon L, Carroll RS, et al. Bio-printing of collagen and vegf-releasing fibrin gel scaffolds for neural stem cell culture. Exp Neurol 2010;223:645–52.
[288] Moon S, Hasan SK, Song YS, Xu F, Keles HO, Manzur F, et al. Layer by layer three-dimensional tissue epitaxy by cell-laden hydrogel droplets. Tissue Eng Pt C Meth 2010;16:157–66.
[289] Jakab K, Norotte C, Damon B, Marga F, Neagu A, Besch-Williford CL, et al. Tissue engineering by self-assembly of cells printed into topologically defined structures. Tissue Eng Pt A 2008;14:413–21.
[290] Smith CM, Christian JJ, Warren WL, Williams SK. Characterizing environmental factors that impact the viability of tissue-engineered constructs fabricated by a direct-write bioassembly tool. Tissue Eng 2007;13:373–83.
[291] Mironov V, Prestwich G, Forgacs G. Bioprinting living structures. J Mater Chem 2007;17:2054–60.
[292] Arai K, Iwanaga S, Toda H, Genci C, Nishiyama Y, Nakamura M. Three-dimensional inkjet biofabrication based on designed images. Biofabrication 2011;3:034113.
[293] Kim G, Son J, Park S, Kim W. Hybrid process for fabricating 3d hierarchical scaffolds combining rapid prototyping and electrospinning. Macromol Rapid Comm 2008;29:1577–81.
[294] Sun W, Starly B, Nam J, Darling A. Bio-cad modeling and its applications in computer-aided tissue engineering. Comput Aided Des 2005;37:1097–114.
[295] Lian Q, Li DC, Tang YP, Zhang YR. Computer modeling approach for a novel internal architecture of artificial bone. Comput Aided Des 2006;38:507–14.
[296] Hollister SJ, Lin CY. Computational design of tissue engineering scaffolds. Comput Method Appl M 2007;196:2991–8.
[297] Lacroix D, Planell JA, Prendergast PJ. Computer-aided design and finite-element modelling of biomaterial scaffolds for bone tissue engineering. Philos T R Soc A 2009;367:1993–2009.