Review Article

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Advances in the design of macroporous polymer scaffolds for potential applications in dentistry

Sidi A. Bencherif[1,2,*], Thomas M. Braschler[1,3], Philippe Renaud[3]

[1]School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA
[2]Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA, USA
[3]Laboratory of Microsystems, STI-LMIS4, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

A paradigm shift is taking place in medicine and dentistry from using synthetic implants and tissue grafts to a tissue engineering approach that uses degradable porous three-dimensional (3D) material hydrogels integrated with cells and bioactive factors to regenerate tissues such as dental bone and other oral tissues. Hydrogels have been established as a biomaterial of choice for many years, as they offer diverse properties that make them ideal in regenerative medicine, including dental applications. Being highly biocompatible and similar to native extracellular matrix, hydrogels have emerged as ideal candidates in the design of 3D scaffolds for tissue regeneration and drug delivery applications. However, precise control over hydrogel properties, such as porosity, pore size, and pore interconnectivity, remains a challenge. Traditional techniques for creating conventional crosslinked polymers have demonstrated limited success in the formation of hydrogels with large pore size, thus limiting cellular infiltration, tissue ingrowth, vascularization, and matrix mineralization (in the case of bone) of tissue-engineered constructs. Emerging technologies have demonstrated the ability to control microarchitectural features in hydrogels such as the creation of large pore size, porosity, and pore interconnectivity, thus allowing the creation of engineered hydrogel scaffolds with a structure and function closely mimicking native tissues. In this review, we explore the various technologies available for the preparation of macroporous scaffolds and their potential applications.

Keywords: Hydrogels, Polymers, Tissue engineering.

INTRODUCTION

Pore size and porosity are of paramount importance in the design of biomaterial scaffolds for tissue engineering applications. Pore characteristics play an important role in directing tissue formation and function [1,2]. A substantial amount of scaffold porosity and adequate pore size are usually required for homogeneous cell distribution and interconnection throughout engineered tissues. In addition, increased porosity can have a beneficial effect on the diffusion of nutrients and oxygen, especially in the absence of a functional vascular system [3]. Among the diversity of scaffolding systems available, hydrogel remains a popular choice for a number of biomedical applications. Hydrogels are ideal materials for three-dimensional (3D) tissue scaffolds that mimic the extracellular matrix (ECM). They were the first biomaterials designed for use in the human body because of their similarities with natural soft tissues, excellent biological performance, and inherent cellular interaction capability.

Hydrogels are crosslinked macromolecular networks formed by hydrophilic polymers swollen in water or biological fluids [4-20]. Hydrogels may be prepared in various ways. These in-

clude one-step procedures like polymerization and parallel crosslinking of multifunctional monomers, as well as multiple step procedures involving synthesis of polymer molecules with reactive groups and their subsequent crosslinking [14,15]. Homogeneous hydrogels have been used widely in various applications, especially in the controlled drug delivery area where characteristics that limit diffusion are required [14-20]. A significant body of work also exists exploring the suitability of hydrogels in tissue engineering for regenerative dentistry and oral reconstruction. However, the use of hydrogels in tissue engineering has not avoided the persistent issue of insufficient vascularization [21]. Approaches used in an attempt to improve scaffold vascularization include the introduction of macropores [22]. Vascularization is crucial for the development and the repair of most tissues, and is a precondition for the healing of bone defects [23].

When designing scaffolds for any tissue engineering application, a major consideration is the pore size. It has been accepted that pore structure is an essential parameter in the development of scaffolds and that pores must be interconnected and large enough to allow for cell growth, the adequate supply of nutrients, the prompt removal of metabolic by-products, vascularization, and new tissue formation and remodeling so as to facilitate host tissue integration upon implantation. It is thus crucial for the remodeling of bone substitutes and their replacement with osseous tissue. Furthermore, pore volume (porosity) and pore size, shape, and distribution also should be considered [24]. A previous study demonstrated that permeability increases with increasing pore size due to a reduction in specific surface area [25]. If pores are too small, cell migration is limited, resulting in the formation of a cellular capsule around the edges of the scaffold [26]. This in turn can limit the distribution of nutrients and removal of waste products, resulting in necrotic regions within the construct. As a result, the mean pore size within a scaffold affects cell adhesion and ensuing proliferation, migration, and infiltration [27]. A number of 3D porous scaffolds fabricated from synthetic or naturally derived biodegradable polymers have been developed and used for liver, bladder, nerve, skin, bone, cartilage, and ligament tissue engineering, and more recently, regenerative dentistry [28,29].

In contrast to biomaterial scaffolds with low void volumes, higher porosity and pore size result in greater tissue ingrowth *in vitro* and *in vivo* [30]. However, this trend results in diminished mechanical properties, thereby setting an upper functional limit for pore size and porosity. Thus, a balance must be reached depending on the repair, rate of remodeling, and rate of degradation of the scaffold material. Hydrogels are usually prepared by a solution polymerization technique, which entails polymerizing monomers in a suitable solvent
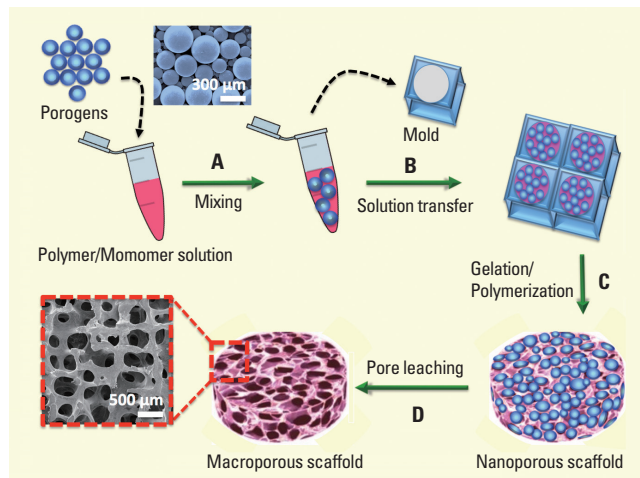

Figure 1. Illustration of the fabrication steps of macroporous hydrogel scaffolds using sacrificial porogens. (A) Degradable particles are collected and dispersed in a crosslinkable prepolymer solution. (B) After mixing, the porogen-containing suspension (here, the porogen consists in microspheres) is poured into mold prior to solidification. (C) Gelation occurs during the polymerization while encapsulating leachable porogens. (D) Porogen removal via controlled degradation gives rise to a three-dimensional porous network with interconnected macropores and macrochannels.

[11-18]. The nature of a synthesized hydrogel, whether a compact gel or a loose polymer network, depends on several factors, including the type of monomer, the amount of diluent in the monomer mixture, and the amount of crosslinking agent. As the amount of diluent (usually water) in the monomer mixture increases, the pore size also increases up to the micron range [31]. Hydrogels with effective pore sizes in the 10 nm–10 μm range are termed microporous, while hydrogels having pores above 10 μm are usually called macroporous or superporous [32]. There are a number of ways in which macroporous hydrogels can be fabricated, most commonly relying on physical interruptions in the hydrogel-forming solution, which is removed after gelation (Fig. 1).

These physical interruptions, known as porogens or pore forming units, are leached out postgelling to create the desired pores. Interconnections are formed in regions where porogens have come into close proximity with one another. A range of materials has been used as porogens including salt, sugar, silica, gelatin spheres and even live bacteria [33-37]. The benefits of large interconnected porosity include improved cell seeding as well as channels to guide cell migration and ingrowth proliferation, provide minimal diffusional constraints during culture, and allow even spatial cell distribution throughout the scaffold to facilitate homogeneous tissue formation. For instance, cell guidance is important for bone regeneration around dental implants using tissue-engineered constructs.

Since the properties of hydrogels can be controlled with the synthesis conditions, new fabrication techniques have recently emerged to generate scaffolds with well-defined macroporous structures while retaining gel integrity. Several methods have been developed to prepare these kinds of porous 3D biodegradable scaffolds, including gas foaming, fiber extrusion and bonding, 3D printing, phase separation, emulsion freeze-drying, and porogen leaching [28].

Given that few biomaterials possess all the necessary characteristics to perform ideally, researchers have pursued the development of hybrid or composite biomaterials to synergize the beneficial properties of multiple materials into a superior matrix. In the context of regenerative dentistry, one strategy to promote the formation of healthy tissue involves the combination of natural and synthetic polymers with various other materials to enhance cellular interaction, encourage integration into host dental tissue, and provide tunable material properties and degradation kinetics [38]. For instance, the addition of inorganic materials (e.g., bioceramic glasses) to a polymer scaffold has several advantages, including combining the osteoconductivity and bone-bonding potential of the inorganic phase with the porosity and interconnectivity of the 3D construct.

This short review aims to summarize the state-of-the-art technologies available for the preparation of macroporous

Figure 2. Tissue engineering is a promising therapeutic strategy that combines cells, biomaterials, and microenvironmental factors to induce differentiation signals into transplantable formats and promote tissue repair and/or functional restoration. One major approach to tooth regeneration is based on tissue engineering, which consists of seeding isolated tooth cells on macroporous scaffolding materials *in vitro* along with biologic therapeutics (e.g., growth factors, genes) and subsequently implanting them into the host site. Dental regeneration includes the repair of the entire tooth structure (enamel, dentin, and pulp), periodontal tissue (cementum and ligament), alveolar bone, blood vessels, and nerves.

polymeric scaffolds with well-defined structural features and their potential biomedical applications, especially in the context of tissue engineering approaches for regenerative dentistry (Fig. 2). In the field of dentistry, tissue engineering may be applied to different types of tissues related to the oral cavity, including bone, cartilage, skin and oral mucosa, dentin and dental pulp, and salivary glands.

POROGEN LEACHING

Porogen leaching, also known as solution casting/particulate leaching, has been a widely used and simple technique for the creation of porous scaffolds in tissue engineering. This method usually involves mixing water-soluble salt particles into a biodegradable polymer solution. The mixture is then cast into a mold of the desired shape. After the solvent is removed by evaporation or lyophilization, the salt particles are leached out to obtain a porous structure. This method has the advantages of simple operation and adequate control over pore size and porosity by the salt/polymer ratio and particle size of the added salt. Various porogens, including sodium chloride, sugar crystals, gelatin, and polymers, have been successfully used to fabricate porous structures [37,39]. However, the wide variations of pore sizes, lack of interconnectivity, and irregular pore geometry have limited the use of this type of porogen in current tissue engineering applications. Further, the difficulty of removing soluble particles from the interior of a polymer matrix makes it difficult to fabricate very thick 3D scaffolds. Recently, different strategies have been used to address some of these issues. A new generation of porogen has been developed using the principles behind nucleation and crystallization science [40]. Also, paraffin spheres have been used as pore-generating materials to create biodegradable polymer scaffolds with spherical pore shape and well-controlled interpore connectivity. These recent technologies provide a simple, convenient, more precise, and cost-effective method over traditional techniques of generating porous macrostructures.

Recently, gelatin-based bioactive glass hybrid scaffolds with a robust interconnected macroporous structure have been prepared using pore leaching technology [41]. In this work, 3D nano-fibrous gelatin/silica bioactive glass hybrid scaffolds that mimic the nano-structured architecture and chemical composition of a natural dental ECM were used for the enhancement of odontogenic differentiation and biomineralization of human dental pulp stem cells. The leaching process may, therefore, provide great freedom in designing versatile scaffolds for dental tissue-engineering applications.
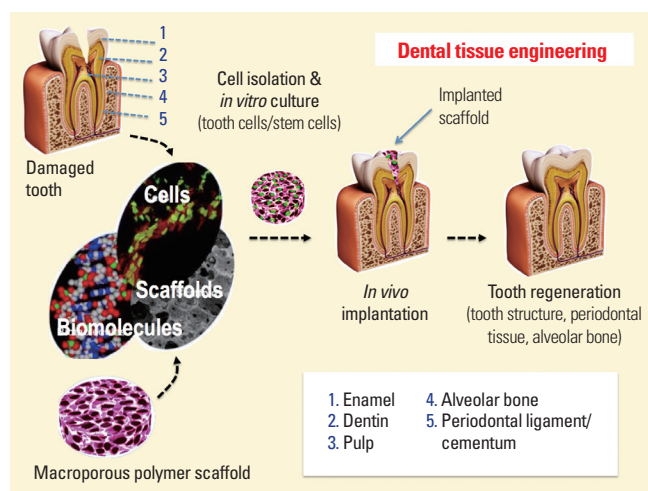
THE EMULSION FREEZING/FREEZE-DRYING TECHNIQUE

The emulsion freezing/freeze-drying technique, due to its usefulness for creating high-porosity scaffolds and controlling pore size, has been employed to fabricate porous tissue engineering scaffolds [42,43]. Highly organized 3D porous structures can be obtained if processing parameters are carefully selected and used. An interconnected porous structure can be fabricated with pore sizes ranging from several microns to a few hundred microns with porosities up to 90% [44]. This method has the added benefit of being amenable to the incorporation of protein-based growth factors at the time of processing [28].

This method consists of creating an emulsion by homogenization of a polymer solvent solution and water, rapidly cooling the emulsion to lock the liquid state structure and removing the solvent and water by freeze-drying. The use of ice particulates as the porogen material has enabled replacement of the conventional method of porogen leaching by repeated washing with water, with freeze-drying. The porogen removal is easier and more complete. The degree of interconnection within the scaffolds increased as the weight fraction of the ice particulates increased. The polymer concentration also had some effect on the pore wall structure; specifically, lower polymer concentration resulted in more porous pore wall structures. The porosity and surface area/weight ratio increased with the increase of the weight fraction of the ice particulates. Therefore, the pore structure of the porous temporary scaffolds could be manipulated by varying the shape, weight fraction, size of the ice particulates, and the polymer concentration [42-44].

It was reported that the scaffold degradation profiles varied according to the type and molecular weight of the polymers and biocompatibility demonstrated that the scaffolds were nontoxic and osteoprecursor cells seeded into scaffolds exhibited the ability to attach, propagate, and differentiate into a calcified structure [45]. In comparison to solvent casting/particulate leaching, the scaffolds produced with this method offer higher specific pore surface area as well as the ability to make thick polymer scaffolds.

Various growth factors could also be incorporated into the porous polymer scaffold by choosing appropriate solvents to dissolve the biodegradable polymer, which will not denature growth factors. The development of this type of porous biodegradable material for use as scaffolding for the sustained 3D growth of tissue has been a fast growing field that has gained interest in bone tissue engineering and may also have specific applications for oral and periodontal regeneration [46].

INVERSE OPAL HYDROGELATION

Within the last two decades, researchers have recognized that colloidal particles could be used as templates for a new class of three-dimensionally ordered macroporous (3D-OM) materials (Fig. 3) [47,48].

This templating process is adaptable to producing films, monoliths, rods, and even hydrogels, depending on the material composition and the specific preparation method [48]. The resulting macroporous products are composed of solid thin walls that surround close-packed spherical voids with a diameter varying from the submicron to millimeter range (Fig. 2). This geometry has been described as an inverse opal, since it is the inverse replica of the close-packed spherical colloids that constitute natural opal gemstones. Researchers worldwide have responded to this development with stunning breakthroughs that combine this ordered nanoarchitecture with diverse chemical compositions. A variety of potential applications have been investigated for materials built upon inverse opal structures, ranging from applications that benefit from the periodic structures (e.g., photonic crystals and optical sensors) to those that benefit from easy access to the relatively large surface areas provided by the interconnected macropores (e.g., tissue regeneration, immunotherapy). Bioactive glasses derived from inverse opal structures (3D-OM BG) have been investigated in detail and their biocompatibility verified [49-51]. Cell culture studies of 3D-OM BG with osteoblastic cells indicated that leachates from 3D-OM BG particles are nontoxic, that the 3D-OM BG particles themselves are not cytotoxic, and that they are compatible with these cells *in vitro* [50,51]. The cells could attach, spread, and proliferate on and around 3D-OM BG particles. These studies indicate the viability of using 3D-OM BG as a novel bone filler.

Macroporous hydrogels with inverse opal structures also provide suitable scaffolds for cell growth and tissue engineering. Colloidal particles were used to template 3D-OM polyacrylamide hydrogels with interconnected voids on the order
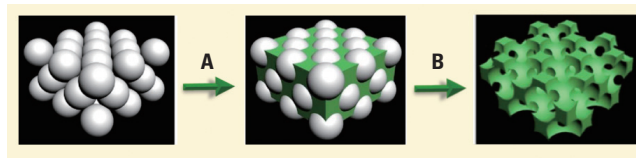

Figure 3. General steps in the preparation of three-dimensionally ordered macroporous (3D-OM) scaffolds by colloidal particle templating. The concept of preparing 3D-OM materials is simple: close-packed colloidal particle are infiltrated with a solution or vapor-phase chemical precursors (A), followed by polymerization and removal of templates by thermal processing, solvent extraction, or chemical etching (B).

of 100 μm, which acted as 3D cell scaffolds for tissue growth and regeneration [48]. The inverse opal architecture led to much greater swelling ratios and swelling kinetics than in bulk hydrogels. Cell culture experiments with two human cell lines demonstrated that the 3D-OM hydrogel scaffold facilitates infiltration, and that cells remain viable within the structure, thereby enabling tissue regeneration. The transparent nature of the hydrogel allowed for continuous high-resolution optical monitoring of cell proliferation and cell-cell interactions within the scaffold.

CRYOGELATION

Cryogels, for use in the fields of tissue engineering and regenerative medicine, are gaining increased interest due to their inherent interconnected macroporous structure and ease of formation in comparison to other macropore forming techniques [52]. Cryogelation is a simple method that avoids the need for porogen removal and forms inherently interconnected scaffolds [52]. For these reasons, cryogels are becoming one of the macroporous hydrogels of choice as indicated by the steadily increasing number of publications on the topic [5,52]. Cryogels, characteristically sponge-like macroporous hydrogels formed at temperatures below the freezing point of the solvent (e.g., water), avoid concerns such as cytotoxicity by utilizing frozen solvent crystals as the interconnecting porogen. Porogen removal is achieved by simply holding the cryogel at temperatures above the solvent freezing point [52]. Typically, the gel solution is cooled below the freezing point. At these temperatures, a large percentage of the solvent crystallizes; however, a portion of the gel solution is maintained in its liquid form. As the solvent crystallizes, the hydrogel constituents are concentrated in liquid microphases, rather than preserved in the crystallized macrophase. The concentration of the hydrogel monomers, oligomers, or polymers is known as cryoconcentration and accelerates the rate of gel formation [53]. After a suitable gelation period, the cryogel is returned to room temperature. As the solvent crystals melt, hydrating the network, an interconnected macroporous hydrogel structure is formed (Fig. 4). For tissue engineering applications, it is desirable that the pores within hydrogels are highly interconnected to allow for the migration and proliferation of cells and to limit regions of inaccessibility. The necessity for pore interconnectivity highlights a significant advantage of the cryogel technique over other pore-forming techniques, as cryogels are inherently highly interconnected. During solvent crystallization, solvent crystals grow until the leading edge comes into contact with another crystal front, resulting in highly interconnected porous gels [54].

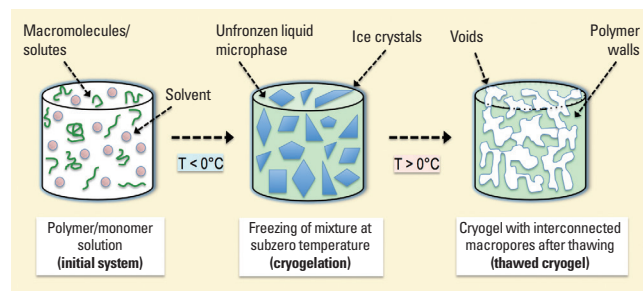In the application of cryogels in tissue engineering and as a

Figure 4. Cryogelation process: monomers and/or polymers are mixed in an aqueous solvent and then the whole system is incubated at subzero temperatures for cryopolymerization and/or gelation, along with ice crystal formation; the ice crystals function then as a porogen during polymerization. After complete gelation, and when incubated at room temperature and washed with water to remove unreacted monomers or polymeric precursors, the ice crystals melt and leave behind large interconnected macropores.

cell support construct, there is a focus on naturally occurring materials such as chitosan, alginate, gelatin, and collagen, due to their biocompatible nature and the existence of well-documented crosslinking methods. Cryogels have been and continue to be investigated for a number of bio-related applications, including bioseparation, biocatalysis, chromatography, monolayer cell proliferation, and more recently, regenerative medicine [55-57]. Cryogel scaffolds have the potential to be utilized in a wide range of tissue engineering applications, reaching from cartilage to neural tissue repair. Due to the cryoconcentration of polymers, cryogels generate very robust porous sponge-like gels, which makes them appropriate scaffolds for cartilage and nonload bearing bone applications [57]. *In vitro* studies of cryogels for cartilage tissue engineering showed that cryogels showed ideal mechanical properties in addition to appropriate open porous morphology that allowed for chondrocyte infiltration and proliferation [57,58]. Recently, in *in vivo* cranial defect model studies, hydroxyapatite (osteoconductive component of bone)-based cryogels exhibited osseous tissue integration within the implant and mineralized functionally stable bone restoration [59]. Evidently, cryogel scaffolds have the potential to be utilized for a wide range of tissue engineering applications, reaching from cartilage to dental restoration.

ELECTROSPINING/FIBER EXTRUSION AND BONDING

Several fabrication techniques have been developed and advanced to fabricate fibrous porous scaffolds with controlled 3D pore structure include electrospinning, textile processing of extruded polymer fibers, bonding of nonwoven meshes, and melt spinning [60]. Fiber meshes consist of individual fi-

bers, knitted or randomly deposited, resulting in 3D patterns of variable pore size. To produce the fibers, one can distinguish the traditional extrusion and spinning techniques from the more recently developed electrospinning.

Traditional wet or melt exclusion can be applied to many polymers, both synthetic and natural, and also metals and hydrogels, and results typically in fiber and pore sizes on the order of tens to hundreds of microns. Traditional spinning devices range from cotton candy machines and syringe injection into an agitated or rotating bath to fully automated industrial production lines. The traditional fiber meshes made from many materials have been investigated as macroporous scaffolds, since the pores are intrinsically interconnected and on suitable length scales for tissue ingrowth. Polymer-based fiber scaffolds, shaped with polyglycolide (PGA), poly(lactic-co-glycolic acid) (PLGA), and poly(l-lactide) (PLLA) fibers, have been investigated for cell transplantation and regeneration of various tissues such as nerve, skin, esophagus, muscle, ligament, bladder, and cartilage [61].

Hydrogel-based fiber meshes have, for instance, been produced by extrusion of alginate, chitosan, collagen, and silk [62-65]. Chitosan, collagen, and silk naturally support cell adhesion, while alginate requires specific modification for surface cell attachment but allows cell entrapment during the extrusion process. Without particular fiber bonding techniques, randomly arranged fiber meshes show little shape memory, since the fibers can slide relative to each other. For this reason, fiber bonding methods have been developed.

Electrospun meshes have gathered interest in tissue engineering applications in the last decade [66-68]. In electropinning, an electric field is used to drive the extrusion of a polymer filament from a droplet suspended from a needle or die. This leads to the production of micron- and submicronscale fibers, such that electrospun meshes are characterized by feature sizes that are much smaller than in traditionally extruded meshes. Electrospun meshes have been shown to support cell attachment, alignment, proliferation and differentiation [69,70]. In the context of tissue engineering, however, the small pores limit *in vitro* cell infiltration as well as *in vivo* tissue ingrowth and vascularization within the bulk of the electrospun scaffold (ES), as these cellular and tissue ingrowth processes require pore sizes of up to 500 μm [67,71-73]. Consequently, cells often remain on the surface of ES and host tissue responses are limited to the periphery of implanted scaffolds [73,74]. To overcome this limitation, several variations of conventional electrospinning to increase the scaffold pore size have been reported. These techniques include the use of porogens between electrospun fibers as well as cryogenic electrospinning with *in situ* formation of ice crystals as porogens [74,75]. An interesting approach offering nearly ar-

bitrary control over the fiber disposition and thus pore size is termed electrospinning with direct writing [76]. In this technique, the fibers are collected on a moving stage, with the speed of the collector being matched to the fiber extrusion rate such that the fibers are deposited straight and at controlled positions rather than randomly. An increased pore size indeed promotes *in vivo* cellular infiltration and vascularization, thereby overcoming the limitations of conventional ES [75,77]. There are also techniques termed cell electrospinning and microintegration that incorporate cells within the scaffold during electrospinning [78-81].

Both traditional extrusion and electrospinning are simple processes, one of their main benefits being the fact that they are easily scalable to potentially very large production volumes; electrospinning allows the production of smaller fibers, although this is not necessarily advantageous in itself for tissue engineering purposes.

GAS FOAMING

Macroporous foams prepared by a gas-foaming technique are commonly used for fabrication of tissue engineering scaffolds due to their potentially high interconnectivity and good mechanical properties. The gas-foaming technique uses the formation of gas bubbles in a prepolymer to create the scaffold pores, followed by curing of the prepolymer leading to the solidification of the foam. The gas bubbles can be created either by a chemical reaction within the prepolymer, by changing the physical conditions leading to a decrease of the gas solubility in the liquid and subsequently gas bubble nucleation, or by bubbling of an inert gas [82]. Typically, water soluble polymers such as alginate, gelatin, poly(ethylene glycol) (PEG), dextranes, or acrylates are used with chemical gas formation reaction or bubbling techniques, while hydrophobic polymers such as PLLA, PLGA, and PGA are more commonly foamed by dissolving supercritical CO_2 in them followed by gas bubble formation upon pressure reduction [82].

A possible problem in gas foaming techniques is the formation of skin layers between adjacent bubbles, leading to poor interconnectivity despite high pore volume fractions. To improve the pore structure, one possibility is to integrate salt crystals into a hydrophobic polymer mass prior to the supercritical gas foaming step; leaching of the salt crystals in water after the gas foaming step leads to an open-pore structure. For other protocols, the problem of remaining skin layers does not seem to arise. For instance, PEG diacrylate gels foamed with ammonium bicarbonate, and also alginate gels foamed using a bicarbonate/acid system, show full interconnectivity without the need for any particular precaution [83,84].

Gas foaming techniques can be carried out with a wide va-

riety of materials, and in some cases very easily on a lab scale by using commonly available blowing agents such as sodium or ammonium bicarbonate. It is, however, at this point not always clear why, in certain systems, proper pore interconnectivity is achieved without further problems, while in others, sophisticated process improvements are needed.

3D PRINTING & PHOTOLITOGRAPHY

Macroporous scaffolds are also increasingly being fabricated by top-down processes such as 3D printing and photolithography. These techniques allow full control over the distribution and size of pores and wall material in nearly arbitrary geometries, and therefore hold great promise in terms of organ-specific 3D design and fabrication [85]. They, however, depend on specific installations, and may also impose some limits on the materials that can be structured.

In inkjet printing, a liquid precursor solution is ejected droplet-wise from a nozzle and collected on a substrate, where it solidifies. By specifying layer-by-layer where droplets of a given ink should be printed, one can build nearly arbitrarily complex structures with a resolution given by the droplet size, but typically on the order of tens of microns. Several variants of the technique exist: historically, a first main implementation of 3D printing was to deposit binder into sequential powder layers, followed by removal of the unbound powder; more recently, the focus has switched to polymer extrusion, and also to cell and hydrogel printing [86-88].

As the use of inkjet printing to produce hydrogel scaffolds is relatively novel, a substantial amount of work is still required to increase the choice of printable inks with biologically meaningful composition for many hydrogel systems; one of the main difficulties lies in finding formulations that avoid feature deterioration before solidification can occur [85]. A possible strategy to achieve this goal is to increase the viscosity of the printing solution on the one hand, and use photoactivated crosslinking on the other, to accelerate polymerization; another is to mix a rapidly gelling component, such as alginate, with biologically more relevant, but more slowly gelling components [88,89]. Another challenge consists in obtaining hollow structures; in most cases, this requires the use of a sacrificial material such as gelatin or agarose, adding complexity to the process [87,90]. However, since 3D printing is rapidly becoming cheaper and more accessible, one can expect substantial developments and a rapid trend towards better solutions, namely for the preparation of the inks.

Photolithography, like inkjet printing, is a top-down technology allowing the specification of nearly arbitrary scaffold geometries. It uses specific illumination patterns to induce polymerization at desired places, such that upon leaching of an unreacted monomer, the desired structure is obtained. Production of hydrogel scaffolds by means of photolithography has been mainly based on the photopolymerization of small acrylic molecules or acrylated hydrogel prepolymers such as PEG diacrylate and acrylated gelatin, alginate, or other macromolecules [91-95]. To obtain 3D structures, two main techniques are currently available. The first one is stereolithography, in which the photoresin is exposed layer by layer with an appropriate pattern at each level [96]. The second uses two-photon exposure, allowing the exposure of small voxels in the center of a laser focus within a 3D volume without initiating polymerization on the remaining light path [97].

To sum up, the top-down approaches allow unprecedented control over the exact gel pore size and geometry, but do require investment in specific equipment, and for a given hydrogel system, oftentimes also some chemical development to achieve suitable control over polymerization rates and rheology. Currently, whilst bioprinting is still at the primary stage of development, it is believed that this technology holds great promise in tissue engineering including tooth regeneration [98-100].

CONCLUSION

Regenerative therapy in dentistry is limited by both the body's natural capacity for regeneration and the materials and methods currently available. To overcome these limitations, the field of dental tissue engineering is promising, but there exist major challenges that must be met in the near future for this new field to reach its potential for application. Tissue engineering will likely have its most significant impact in dentistry via bone tissue engineering and regeneration.

Although the ideal 3D matrix materials for dental bone tissue engineering have yet to be developed, much progress has been made during the last few years. The requirements of scaffolds for dental bone tissue engineering are complex. A variety of characteristic parameters, such as the degradation rate, mechanical strength, porosity, pore size, pore microstructures, surface chemistry, hybridization with inorganic materials, and topography, should be carefully considered and controlled for the design and fabrication of scaffolds to meet the needs of this particular tissue engineering application. The development and fabrication techniques of novel biodegradable biomaterials and scaffolds with well-defined macrostructures reviewed will constitute a centerpiece of the research efforts in the field of regenerative medicine in dentistry. Using polymer scaffolding to controllably manipulate cell function and the spatiotemporal release of biologic ther-

apeutics will likely enable development of new therapies and technologies for dental bone tissue repair and regeneration as well as other oral health applications.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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