Published in final edited form as:

Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2014 March ; 6(2): 136–154. doi:10.1002/wnan.1248.

Injectable Foams for Regenerative Medicine

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Abstract

The design of injectable biomaterials has attracted considerable attention in recent years. Many injectable biomaterials, such as hydrogels and calcium phosphate cements, have nanoscale pores that limit the rate of cellular migration and proliferation. While introduction of macroporosity has been suggested to increase cellular infiltration and tissue healing, many conventional methods for generating macropores often require harsh processing conditions that preclude their use in injectable foams. In recent years, processes such as porogen leaching, gas foaming, and emulsion-templating have been adapted to generate macroporosity in injectable calcium phosphate cements, hydrogels, and hydrophobic polymers. While some of the more mature injectable foam technologies have been evaluated in clinical trials, there are challenges remaining to be addressed, such as the biocompatibility and ultimate fate of the sacrificial phase used to generate pores within the foam after it sets *in situ*. Furthermore, while implantable scaffolds can be washed extensively to remove undesirable impurities, all of the components required to synthesize injectable foams must be injected into the defect. Thus, every compound in the foam must be biocompatible and non-cytotoxic at the concentrations utilized. As future research addresses these critical challenges, injectable macroporous foams are anticipated to have an increasingly significant impact on improving patient outcomes for a number of clinical procedures.

Introduction

Minimally invasive surgical techniques have attracted considerable interest in the past few decades, as supported by the increasing number of publications related to the topic (Figure 1). Minimally invasive surgeries (MIS) are currently practiced in a variety of operative procedures, including orthopaedic, vascular, and neurological¹. There is still a lack of evidence supporting the idea that MIS provide a better healing outcome than conventional open procedures². However, retrospective studies comparing results of open and minimally invasive surgeries associate MIS with lower exposure of deep tissues to pathogens, reduced disturbance of the skin and skin flora, and reduced operative site dead space (a space created

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Conflict of interest: Vanderbilt University has licensed the injectable polyurethane foam technology co-invented by the authors (EMP, AJH, and SAG) and reviewed in this article to Medtronic Sofamor Danek (Memphis, TN). The authors are collaborating with Medtronic on commercial development of the technology as an injectable and settable bone void filler.

between tissues that are normally connected, where fluid can accumulate and promote infection)³. Consequently, suggested advantages of MIS include shorter hospitalization periods¹ as well as reduced pain and risk of post-operative infection for the patient. In contrast to open procedures, access to the region of interest using MIS is achieved through small incisions to the body. Surgeons use these incisions to introduce endoscopes with imaging devices and surgical instruments to the wound site. The small incisions are also the entry point for biomaterial scaffolds for the treatment of tissue defects. Thus, biomaterials for MIS must be able to adapt to the size and geometry of the endoscopes or needles through which they are delivered. Injectable biomaterials offer this capability, and can also conform to complex defect geometries. Tissue engineering efforts to develop injectable biomaterials have increased at an exponential rate since 1980, as evidenced by the plot of the number of publications since 1980 shown in Figure 1. While not all MIS procedures require an injectable scaffold, it is interesting to note that the number of publications on MIS follows a similar trend (although the number of publications on MIS is an order of magnitude greater than that on injectable biomaterials). This observation suggests that the demand for injectable biomaterials has been driven at least in part by the growth in MIS, and will continue to grow as surgeons develop minimally invasive procedures to reduce patient discomfort.

Injection of flowable precursors that set by chemical reaction or physical gelation to form a macroporous scaffold *in situ* presents considerable challenges. Injectable biomaterials are generally administered as viscous liquids that cure *in situ*; thus, prior to setting, the material must be amenable to flow through a small-bore needle. Ideally, the materials should conform to complex tissue defects, achieve intimate contact with surrounding tissues, and set after clinically relevant gelation times to form a scaffold with interconnected macropores ($>10\ \mu\text{m}$) and mechanical properties approaching those of the host tissue⁴. In addition, injectable scaffolds must be non-cytotoxic, support cellular infiltration, degrade to non-cytotoxic breakdown products, and promote balanced remodelling (i.e., the rate of new tissue formation is comparable to the rate of the scaffold degradation). Injectable hydrogels and calcium phosphate cements with nano- or micro-structured pores have been investigated extensively for tissue engineering applications. Since the pores are generally smaller than the cells, cellular infiltration is impaired, and consequently the materials must first degrade in order for cells to infiltrate the scaffold and deposit new tissue. The presence of interconnected macropores has been reported to enhance healing by facilitating diffusion of nutrients, cellular proliferation and migration, and deposition of new extracellular matrix⁵. Thus, in recent years, injectable foams with macropores ($>10\ \mu\text{m}$) have attracted interest in response to the need for injectable materials that more rapidly infiltrate with cells and remodel. This review focuses on the characterization and development of injectable and settable foams exhibiting interconnected macropores, such that the size of the pores is comparable to or exceeds that of the cells. New developments in injectable macroporous foams fabricated from calcium phosphate cements, biphasic hydrogels, hydrophobic polymeric scaffolds, and gas-foamed polymer scaffolds are reviewed. Each section starts with a review of the chemistry of the materials, techniques for processing the materials as injectable foams with interconnected macropores, and applications in preclinical studies. Some of the more mature technologies have been evaluated in clinical trials, and thus translation of injectable foam technology toward commercial products is also reviewed.

CHARACTERIZATION OF INJECTABLE FOAMS

Proper characterization of an injectable foam system encompasses multiple strategies from a variety of design perspectives. Several researchers have begun to quantify the definition of injectability, but related methods and parameters have yet to receive widespread acceptance. At a minimum, a qualitative description, including the needle size and plunger apparatus,

should be provided as characteristics of an injectable foam. In one embodiment, a biomaterial is considered injectable if it can be injected with a force <100 N using an arbitrary syringe size⁶. Alternatively, injectability can be defined as the cannula diameter below which the paste is no longer flowable, which is particularly relevant to MIS⁷. The working time, which is the time required for the material to set, should be in the clinically relevant range of 10 min⁷. Table 1 lists various design perspectives, corresponding parameters, and utilized methods that have been published for injectable foam systems. The list is organized generally as proceeding from fabrication (top) to implementation (bottom). Several methods appear to be accepted as foundational for characterizing injectable foams. Most utilize evaluation of architecture via SEM imaging and/or gravimetric analysis of bulk porosity. A variety of mechanical testing techniques have been implemented, from compression to three-point bending. Finally, *in vitro* evaluation of cell-material interactions is supported by numerous cell viability, proliferation, and differentiation assays.

In the future, as injectable foams move toward clinical application, additional characterization methods should include degradation and biocompatibility of reactive components. The mechanism and degradation rate of injectable foams affects the release of degradation products and the healing of surrounding tissue. Ideally, the degradation rate follows the rate of cellular infiltration, remodelling, and tissue regeneration. Many types of foam are designed to degrade by passive mechanisms (e.g., hydrolysis), but in recent years cell-degradable materials have allowed cells to actively degrade the material by utilizing proteolytically cleavable peptides in the backbone. This process is anticipated to equate the rates of scaffold degradation and tissue restoration. In order to prevent bacterial contamination and subsequent potential infection, proper sterilization techniques that do not alter the properties of reactants and/or foams must be utilized. Fully evaluating the overall biocompatibility of an injectable foam encompasses testing not only the foam as formed *in situ* and its breakdown products, but also each individual component initially present in the injectable formulation. It is important to understand the potential risk of leaching toxic reactants and/or released reaction by-products into the wound bed. Subsequently, characterizing the handling properties of an injectable material will provide insight into the potential for extravasation and spread of reactants to unintended anatomical sites. Relatedly, optimizing reaction conditions to minimize the risk of extravasation, prevent an exotherm that could damage local native tissue, and maximize the conversion of precursors is anticipated to improve the biocompatibility of the injectable foam.

INJECTABLE AND SETTABLE CALCIUM PHOSPHATES

Low porosity calcium phosphate cements—Since their initial discovery in 1982⁸, injectable calcium phosphate cements (CPCs) have been successfully introduced into the clinic for a number of orthopaedic and craniomaxillofacial applications, including repair of tibial plateau fractures and calvarial defects. CPCs have been investigated extensively as injectable bone replacement biomaterials due to their similar chemical composition to the mineral component of bone, biocompatibility⁹, osteoconductivity, and fast setting times (< 5 min)¹⁰. These biomaterials set at a physiological pH with minimal reaction exotherm and do not release toxic monomers or solvents^{11, 12}. Apatite, which has low solubility and resorbs slowly, and brushite, which has higher solubility than apatite and resorbs more rapidly, comprise the two primary classes of CPCs¹³. While CPCs set by an acid/base reaction that can reduce the pH of the paste to values as low as 3¹⁴, a number of *in vivo* studies have reported favorable host responses after setting^{15, 16}. In a recent study, the mechanism of cell-mediated degradation of brushite CPCs was investigated by culturing RAW264.7 cells on the cements *in vitro*.¹⁷ The RAW264.7 cells differentiated to macrophages, multinucleated giant cells, as well as osteoclast-like cells, on the brushite CPCs. SEM analysis of the ultra-structure of osteoclast-like cells revealed characteristics

associated with the osteoclast phenotype, such as formation of a sealing zone and ruffled border. Furthermore, osteoclast-like cells were observed to penetrate deep into the interior of the cements, which suggests that brushite CPCs are demineralized by osteoclast-mediated resorption *in vivo*.

In a promising area of new research, the effects of nanometer-scale calcium phosphate (CaP) crystals on cellular interactions are under investigation¹⁸. Coating of mesenchymal stem cells with CaP nanorods has been reported to exhibit enhanced osteoblastic differentiation and production of extracellular matrix production relative to uncoated cells¹⁹, which suggests that nanoscale injectable CPCs may be desirable carriers for stem cells. However, while nanostructured CaPs enhance osteoblast differentiation, the rate of cellular infiltration into monolithic cements is limited by osteoclast-mediated resorption. This rate of infiltration is relatively slow (i.e., $0.02 \mu\text{m}^3 \mu\text{m}^{-2} \text{day}^{-1}$), in contrast to migration and proliferation of cells through interconnected pores²⁰. Thus, a number of recent studies have investigated new methods for introducing macropores into injectable CPCs to enhance cellular infiltration and remodelling.

Porous calcium phosphate cements—Primary limitations of first-generation low porosity CPCs restricting their use in the clinic include brittle mechanical properties, which lead to low shear strength and fracture toughness; slow degradation *in vivo*²¹; and the small pore size ($0.1 - 10 \mu\text{m}$), which results in slow cellular infiltration and ingrowth of new bone^{9,22}. Both low porosity as well as small pore size in CPCs contribute to slow remodelling and ingrowth of new bone, and have thus been suggested as a root cause for the failure of CPCs in periodontal bone repair²³. Slow remodelling of CPCs has also been suggested to contribute to their failure in cranioplasty applications²⁴. Therefore, recent studies have focused on introducing macropores into CPCs and improving their mechanical properties while preserving their favorable biocompatibility in order to improve their clinical performance.

Interconnected macropores $>10 \mu\text{m}$ are essential for promoting infiltration of cells and ingrowth of blood vessels and new bone, into CPCs^{25,26}, as well as for polymeric scaffolds such as hydrogels⁵. In order to preserve the excellent biocompatibility and osteoconductivity of CPCs, adjuvants added to create macropores must be non-cytotoxic. In one approach, multi-phasic cements have been prepared by combining soluble ceramic particles, such as calcium sulphate, with less soluble particles, such as tricalcium phosphate or hydroxyapatite^{27,28}. A bi-phasic CPC was fabricated by mixing α -tricalcium phosphate (α -TCP), calcium sulphate hemihydrate (CSH), and an aqueous solution containing 2.5 wt% Na_2HPO_4 ²⁹. The resulting cement comprised an apatitic phase (calcium-deficient hydroxyapatite, CDHA) and a soluble phase (calcium sulphate dihydrate, CSD). Figure 2A shows a cement prepared from a mixture initially containing 20 wt% α -TCP and incubated in saline for 14 days. Both small ($\sim 1 \mu\text{m}$) crystals of CSD (labelled D in Figure 2A and viewed at higher magnification in the inset image) and larger residual CSH (labelled H) crystals were observed in the cement. Voids formed by dissolution of the CSH crystals (labelled SH) are also evident in the SEM image. After setting, the soluble CSD and CSH phases dissolved, resulting in pores that facilitated the ingrowth of new bone in a rat on-lay graft model³⁰. The technology has been licensed to BoneSupport and is marketed as CERAMENTTM bone void filler for spinal and orthopaedic indications. Recent clinical studies have highlighted the potential of the partially resorbable biphasic (60% calcium sulphate/40% hydroxyapatite) CERAMENTTM CPC as an effective substitute for non-resorbable poly(methyl methacrylate) (PMMA) for treatment of osteoporotic and traumatic vertebral fractures^{31,32}.

Composites with calcium phosphates—A number of other strategies have been investigated for fabricating injectable macroporous CPCs. Foaming agents such as surfactants³³, hydrogen peroxide solution³⁴, carbon dioxide³⁵, or hydrophobic liquids³⁶, have been utilized to fabricate injectable calcium phosphate foams. In one study, the surfactant sodium dodecyl sulphate (SDS) was used as an air-entraining agent to create macroporous CPCs (Figure 2B)³³. Micro- and macroporosity were controlled by the liquid-to-powder ratio and the concentration of SDS, which stabilized the bubbles formed by the setting reaction of the cement. Alternatively, biocompatible and degradable polymeric microspheres such as poly(lactic-co-glycolic acid) (PLGA)³⁷, poly(trimethyl carbonate), or gelatin^{38, 39} have been investigated to synthesize macroporous CPCs. CPCs incorporating degradable porogens offer the additional advantage of local delivery of drugs or growth factors, since biologics can be encapsulated in the porogens prior to embedding in the calcium phosphate matrix⁴⁰. While incorporation of PLGA microspheres in CPCs has been suggested to improve the initial strength of the composites, the strength decreases upon dissolution of the microspheres and does not increase until new bone starts to grow into the macropores⁴¹. To improve the injectability of CPC incorporating 30 wt% PLGA microspheres, sodium citrate was added to the aqueous solution, which reduced the viscosity of the paste. Due to their macroporosity, injectable CPCs incorporating a porogen have significant advantages compared to monolithic CPCs. Thus, macroporous CPCs are anticipated to be considered a preferred choice for healing of bony defects after regulatory approval²¹. In an alternative approach, CaP granules suspended in aqueous solutions of hydroxy-propylmethyl-cellulose promoted faster initial ingrowth of new bone at the surface of the material, relative to macroporous CPCs^{42, 43}. It has been suggested that the observed early apposition of new bone could potentially enhance the interfacial bonding between host bone and the CPC, thus reinforcing the material for weight-bearing applications²¹.

FABRICATION OF MACROPOROUS POLYMERIC SCAFFOLDS BY POROGEN LEACHING

Macroporous polymeric foams, which can be formed by a number of methods, including gas foaming, solvent casting/porogen leaching, phase-separation, freeze-drying, and electrospinning⁴⁴⁻⁴⁷, have found widespread use in biomedical applications due to the ease of cell and nutrient diffusion into the materials. Typically, macroporous foams are prepared by melting or dissolving polymeric components in a solvent followed by casting in a mold with the desired dimensions for the targeted application^{48, 49}. One of the more easily controlled methods for generating open and interconnected pores in polymeric scaffolds is porogen leaching, in which a sacrificial component is incorporated in the polymer and is subsequently removed by dissolution after the scaffold has been formed⁵⁰⁻⁵². Porogen leaching has been investigated extensively for fabrication of implantable foams due to the relative simplicity of casting the bulk polymer around the porogen in a mold. However, fabrication of porous polymeric foams that can be injected into the tissue defect as a flowable liquid, cure to form a solid scaffold with interconnected pores that facilitate cellular infiltration, and maintain the structural requirements of the native tissue is considerably more challenging. In order to accomplish these objectives, the material must be injected as a low viscosity solution, either neat or dissolved in a non-cytotoxic solvent, that subsequently sets *in situ* by chemical reaction or physical (*e.g.*, thermal) gelation. The utilization of toxic solvents or polymers with high temperature transitions precludes the use of many materials that have been investigated for fabrication of porogen-leached foams. In the following sections, injectable foams prepared by porogen leaching in combination with *in situ* crosslinking reactions or non-toxic solvents are reviewed.

Injectable hydrogels—Injectable hydrogels have been extensively investigated as scaffolds for tissue regeneration, including recent studies with macroporous formulations. Traditionally, hydrogels are composed of a network of hydrophilic polymer chains that are

physically or chemically crosslinked. A common material utilized in biomedical hydrogels is poly(ethylene glycol) (PEG), which can be functionalized with variable moieties and polymerized by photo or thermal initiation. The intrinsic porosity for typical hydrogels is on the length scale of the mesh size of the network (typically 1-100 nm), which is large enough to allow small molecules and proteins to diffuse into the matrix but limits cellular infiltration into the gel since cells are at least two orders of magnitude larger than the size of the pore. Substrates designed to be cleaved by cell-secreted enzymes, such as matrix metalloproteases (MMPs), have been incorporated in the polymer backbone to facilitate cell-mediated degradation and subsequent cellular infiltration of nanostructured hydrogels. Additionally, generating interconnected macropores (>10 μm) within hydrogels is expected to promote cell infiltration throughout the material. As with any porous structure, macroporous hydrogels have inherently lower mechanical strength than their non-porous counterparts; therefore, most of the intended applications are for regeneration of soft tissue. Additionally, the added complexity involving formulation of injectable, macroporous hydrogels limits the available chemistries. Despite these drawbacks, injectable macroporous hydrogels generated by cryogelation techniques^{5, 53} or co-encapsulation of porogens^{54, 55} (e.g., salt crystals⁵² and cell or drug encapsulated beads⁵⁶) have been reported to increase the transport of nutrients, enhance cell viability and migration⁵⁷, and beneficially alter cell differentiation and gene expression^{58, 59}.

Delivery of cells from macroporous hydrogels: The ability to deliver viable cells from injectable biomaterials has been an active area of investigation for several decades⁶⁰, and recent advances in cell encapsulation have enabled the combination of macroporosity and cell delivery for injectable hydrogels. Previous studies reported that human umbilical cord stem cells (hUCMSCs) encapsulated in sodium alginate microbeads maintained high viability when exposed to the harsh reaction, mixing, and injection conditions of a calcium phosphate cement⁶¹. In subsequent studies, degradable alginate-fibrin microbeads (dMB) that released hUCMSCs within 7-21 days were fabricated⁶². These rapidly degrading microbeads were further utilized to encapsulate hUCMSCs in an injectable fibrin scaffold for muscle tissue engineering applications. Fibrin scaffolds augmented with the cell encapsulated dMBs were able to maintain high cell viability compared to the microporous fibrin controls over 16 days⁶³. The macroporous structure that remained after degradation of dMB cell carriers increased gene expression of myosin heavy chain 1 (MYH) and alpha-actinin 3 (ACTN) which are markers of differentiation into muscle fibers⁶³. Additionally, confocal imaging of the cells showed multi-cell fusion into myotubes (Figure 3), further indicating muscle tissue formation⁶³.

As mentioned above, the complexity of formulating a macroporous, injectable hydrogel has limited the types of suitable materials⁶⁴. However, a novel adaptation of a widely utilized biomaterial was recently employed for cell delivery. An alginate-based macroporous hydrogel was generated by cryogelation, as summarized in Figure 4. The cryogels were formed by radical polymerization of methacrylated alginate and RGD-functionalized PEG⁵. During the polymerization the gels are cooled to -20°C at which temperature ice crystals form, partitioning the polymer and generating macropores upon reheating. The foams can withstand >90% deformation and retain >92% of the original shape upon relaxation⁵, which allows the hydrogels to be injected via a 16 gauge needle with little damage. *In vivo* implantations show minimal immune response and the cryogels can be augmented with biomolecules or cells to increase bioactivity⁵. The combination of intrinsic porosity of the alginate and the macroporosity of the cryogel technique increases the proliferative capacity of the implanted cells and further allows native cell populations to infiltrate and integrate within the material.

Injectable fumarates—One of the first systems to employ water-soluble or degradable porogens in an injectable formulation was a copolymer system based on linear polyesters derived from fumaric acid, known as fumarates. This class of biomaterials is known to be highly customizable, biocompatible, and biodegradable. Fumarates contain an unsaturated bond in the monomer backbone that can be utilized to induce *in situ* crosslinking⁶⁵. Specifically, poly(propylene fumarate) (PPF) and similar copolymers have been investigated as scaffolds for regeneration of both soft⁶⁶ and hard⁶⁷ tissue. The low melting point of PPF and its copolymers makes it ideal for injectable applications. In a typical application to synthesize a macroporous PPF scaffold, a soluble porogen is added to a mixture of a methacrylate crosslinker and a fumarate copolymer, which is then reacted *in situ* by light or thermal initiation to form an elastomeric network^{68, 69}. After leaching the soluble porogen, a highly interconnected macroporous structure remains, as shown in Figure 5. Early work with injectable fumarate systems for hard tissue applications utilized sodium chloride microparticles as porogens with the further addition of PLGA microspheres as both a sacrificial porogen as well as a delivery mechanism for therapeutics^{70, 71}. For soft tissue applications, fumarate-based hydrogels have been combined with gelatin microspheres as the degradable porogen to deliver both transforming growth factor β 1 (TGF- β 1) and chondrocytes that maintained high levels of viability over 28 days⁷².

HIGH INTERNAL PHASE EMULSION (HIPE) FOAMS

Emulsion templating has emerged as a foam production technique with high control over pore size, morphology, and interconnectivity⁴⁵⁻⁴⁷. Emulsions used as templates have a droplet volume >74%⁷³ and are known as High Internal Phase Emulsions (HIPEs). As shown in Figure 6, during emulsion templating a monomer (in solution or as a viscous liquid) or a polymer solution is first homogenized with a diluent to create a HIPE. The HIPE is then cast into a mold with appropriate conditions for monomer or polymer densification. After densification, the internal phase can be removed and the resulting foams are known as polyHIPEs⁴⁴. The architecture of the foam is controlled by modifying parameters that influence the stability of the initial emulsion, such as interfacial energy. Adding surfactants has been shown to reduce interfacial energy and thus promote phase separation and droplet size reduction⁴⁶. Interconnectivity of the pores in the resulting foam is related to the thickness of the polymer film between droplets as well as to the densification of the polymer phase. Film thickness depends on the total droplet volume and individual droplet size, with larger droplet volumes generating thinner films. During densification the polymer film shrinks (according to structural characteristics of the polymer) and generates openings between pores in the final foam. As a result, the pore size distribution and interconnectivity of emulsion-templated foams can be modified with variations of surfactant nature and concentration, polymer solution to aqueous phase ratio, and polymer composition^{45, 46, 74}. Even though the understanding and control of polyHIPE stability has increased, the main challenges associated with the application of these emulsions to injectable systems are the need to use biodegradable macromers capable of emulsion formation, as well as chemistries that undergo densification under physiological conditions⁴⁶. Toxicity of the components is also a concern and can be reduced by the use of low viscosity macromers, which eliminates the need for toxic solvents.

An injectable polyHIPE was recently developed using propylene fumarate dimethacrylate (PFDMA) as the polymeric phase⁴⁶. The biodegradable PFDMA macromer was premixed with polyglycerol polyricinolate surfactant (PGPR 4125) and then emulsified with an aqueous solution containing calcium chloride and ammonium persulfate (crosslinking initiator). As shown in Figure 7, the emulsion was then injected into a mold at 37°C and allowed to undergo densification for 12 h. The resulting foam replicated the irregular shape of the mold. After removing the water with vacuum treatment for 24 hrs, the foam had

closed pores with sizes in the range of 4–29 μm , porosity of 75%, and average compressive modulus and strength of 33 and 5 MPa, respectively. When tested *in vitro*, the PFDMA polyHIPE yielded 95% viability of 3T3 Swiss mouse fibroblasts after a 24 hr culture period. Although this formulation has the advantages of being injectable and not using toxic solvents, the authors recognize the need for additional improvements and suggest that ongoing studies are focused on obtaining emulsion-templated foams with open interconnected pores with sizes $>100 \mu\text{m}$. In contrast to the PFDMA polyHIPE that undergoes densification due to radical cross-linking of the methacrylate groups in the macromer, non-covalently cross-linked polyHIPE hydrogels have also been developed using oil-in-water HIPEs⁴⁷. In this case, the aqueous phase contained a thermoresponsive linear polymer (dextran-b-polyNIPAAm) that reversibly aggregated at 37°C and was stable in an aqueous environment (Figure 8A). The resulting foams had open pores with sizes between 1 and 10 μm (Figure 8B), and pore morphology that depended on the conditions under which the emulsion was prepared. The use of a thermoresponsive polymer provided a solid network in the absence of a reactive chemistry. However, its biocompatibility still remains to be tested both *in vitro* and *in vivo*.

POLYURETHANE FOAMS

Polyurethanes (PUR⁷⁵) comprise a diverse family of materials including cast elastomers, thermoplastic elastomers, and flexible foams. Due to their toughness, durability, biocompatibility, and improved biostability, they have been incorporated in a wide variety of implantable biomedical devices. Biodegradable polyurethanes prepared from biocompatible intermediates, such as polyester polyols and aliphatic and lysine-derived polyisocyanates, have attracted considerable interest recently for use as tissue scaffolds and drug delivery systems^{75, 76}. Their ability to be processed by reactive liquid molding renders polyurethanes useful as injectable biomaterials for MIS, in which the material is injected as a reactive viscous liquid that cures *in situ* to form a viscoelastic solid scaffold. Furthermore, macropores that facilitate cellular infiltration can be generated within the scaffold by the process of gas foaming through the reaction of isocyanate groups with water^{77–79} or by encapsulation of porogens⁸⁰.

The synthesis of injectable PUR foams is shown in Figure 9. Component 1 comprises either an isocyanate or an isocyanate-terminated prepolymer, while Component 2 includes all components with active hydrogen groups that react with the isocyanate (NCO) groups. The polyol component, which comprises a hydroxyl-terminated macromer with a polyester, polyether, or polycarbonate backbone, reacts with the prepolymer to form a polymer network linked by urethane groups (the gelling reaction). The NCO groups also react with water, either added to the formulation or present in the wound environment, to yield a di-substituted urea and gaseous carbon dioxide, which acts as a blowing agent to form interconnected macropores within the PUR network^{77–79} as shown in the SEM image (Figure 9). The working time, identified by the G'-G'' crossover point (rheological profile in Figure 9) where the foam transitions from a viscous liquid to an elastic solid, can be tuned to targeted values using urethane catalysts, such as tertiary amines⁸¹ or organometallic compounds. Amine-based catalysts have low cytotoxicity⁸² and selectively catalyze the water-isocyanate blowing reaction⁸³, while the more cytotoxic organometallic catalysts selectively catalyze the polyol-isocyanate gelling reaction. In contrast to the temperatures during cure exceeding 100°C observed for aromatic isocyanates, the exotherm for lysine-derived PUR foams is $<20^\circ\text{C}$ (temperature profile in Figure 9). Other additives, such as stabilizers and pore openers to control miscibility and pore size⁷⁷, matrix particles to control expansion and increase mechanical properties, or biologics to enhance bioactivity⁸⁴, can also be included in component 2. While biodegradable PUR foams have been synthesized from aliphatic polyisocyanates⁸⁵, lysine-derived polyisocyanates have shown

the greatest promise for injectable and settable systems^{78, 79, 81, 83, 86-89}. Reactive PUR scaffolds prepared from lysine-derived polyisocyanates, including lysine methyl ester diisocyanate (LDI) and lysine triisocyanate (LTI), and tertiary amine catalysts, have been reported to induce a minimal inflammatory response on host tissue in both cutaneous and bone defects *in vivo*^{79, 83, 86, 89}. Furthermore, *in vitro* cytotoxicity testing of the leachates from the reactive PUR has shown that the reactive PUR components are non-toxic⁸³. These studies highlight the potential of injectable and settable lysine-derived PURs for both soft and hard tissue regeneration.

Soft tissue applications—LDI^{78, 86} and LTI-derived⁸⁹ prepolymers have been used to synthesize injectable and settable PUR foams for regeneration of cutaneous defects in rodent models. The cured polymers degraded to biocompatible breakdown products; such as lysine, PEG, and α -hydroxy acids⁹⁰; induced a modest and transient inflammatory response; and supported the infiltration of cells and ingrowth of granulation tissue. LTI-derived scaffolds degraded at a rate similar to that of tissue ingrowth, and the regenerated tissue exhibited reduced alignment of collagen, which highlights the potential of the scaffolds for reducing scar formation. The LDI technology has been further investigated in larger animals and clinical trials as a water-curable biomedical adhesive, which is designed to react with hydroxyl and amine groups in the host tissue. LDI-derived TissuGlu[®] Surgical Adhesive (marketed by Cohera Medical) has been approved in the European Union⁹¹, and has been shown to reduce the accumulation of wound exudate in large flap procedures in patients (such as abdominoplasty⁹²) compared to non-degradable cyanoacrylate adhesives.

Bone regeneration applications—PUR biocomposites derived from LDI^{79, 93} and LTI^{81, 94, 95} have been investigated as injectable bone void fillers and cements. An extracellular matrix component, such as demineralized bone matrix (DBM), calcium phosphate particles, or allograft bone particles, is blended with the reactive PUR to increase the osteoconductivity and mechanical properties of the graft and also to reduce its volumetric expansion *in situ*. Lysine-derived PUR composites are injectable, set within clinically relevant working times (e.g., 5-10 min) to form grafts with mechanical strength approaching that of host bone, and remodel and heal to form new bone. In an early study, a four-armed poly(dioxanone-co-glycolide) prepolymer capped with LDI was mixed with calcium phosphate particles in the presence of an diethanolamine catalyst and water to form a reactive putty that expanded to form a foam with interconnected pores⁷⁹. When implanted into the pectoralis muscles of rats for up to 42 days, the putty exhibited a transient inflammatory response that resolved after 3 days. At longer time points, the foams were well integrated with host tissue and showed no evidence of a chronic inflammatory reaction.

In a similar study in bony defects, a settable foam was prepared by reacting an LDI-pentaerythritol prepolymer with a poly(lactide-co-glycolide) polyol (functionality of 4)⁹³. The two reactive liquid components were mixed with stannous octoate catalyst, water, and β -tricalcium phosphate particles (10 μ m) and injected into femoral condyle plug defects in sheep, where they cured in ~10 min and expanded to form porous foam. Defects showed progressive healing from 6 to 24 weeks, characterized by the formation of new cortex, polymer degradation, and appositional bone growth with no signs of acute or chronic inflammation. Cells infiltrated the foam through the processes of migration into open pores as well as polymer degradation. In an alternative approach, injectable foams prepared from an LTI-PEG prepolymer, a poly(ϵ -caprolactone-co-lactide-co-glycolide) triol, allograft bone particles (180 μ m), and the low-toxicity tertiary amine catalyst triethylene diamine were evaluated in femoral condyle plug defects in rats⁸¹ and rabbits⁸³. Compared to LDI prepolymers, the LTI-PEG prepolymer has reduced viscosity (20,000 cSt versus 80,000 cSt for LDI-pentaerythritol) and higher reactivity⁸³. Cells infiltrated the scaffold through migration into open pores, surface degradation of the PUR to non-cytotoxic breakdown

products, and osteoclast-mediated resorption of the allograft bone particles, with no signs of chronic inflammation. While challenges including controlling expansion due to the gas blowing reaction and matching the rate of new bone formation to polymer degradation need to be addressed, the ability of PUR grafts to cure *in situ* without adversely affecting host tissue, degrade to non-cytotoxic breakdown products, and support infiltration of cells and new tissue underscores their potential as injectable foams for tissue repair.

Drug delivery applications—Injectable lysine-derived PUR scaffolds have also been investigated as drug delivery systems to support both diffusion-controlled release of biologics added as labile powders^{95–98} as well as degradation-controlled release of drugs covalently bound to the polymer^{87, 88, 99–101}. The diffusion- and degradation-controlled drug delivery strategies are summarized in Figure 10. Fluorescent images obtained using a DAPI filter (Figure 10A) show uniform distribution of naphthalene compounds in an LDI-glycerol PUR foam, as evidenced by the consistent blue color throughout the material⁸⁸. In contrast, SEM (Figure 10B) and confocal (Figure 10C) images of an LTI-polyester foam augmented with FITC-labeled BSA as a labile powder show discrete BSA-FITC particles distributed throughout the scaffold.

In a recent study, injectable LTI-derived PUR/allograft bone composites augmented with recombinant human bone morphogenetic protein-2 (rhBMP-2) as a labile powder enhanced new bone formation at 6 weeks in a critical-size rabbit calvarial defect model⁹⁴. In other studies, diffusion-controlled release of the antibiotic vancomycin and rhBMP-2 controlled infection⁹⁷ and enhanced bone healing¹⁰² in critical-size defects contaminated with 10⁵ Colony Forming Units (CFUs) *S. aureus*. Release of other biologics, such as nanoparticles delivering siRNA⁹⁸, added to injectable PUR bone grafts as a labile powder has also been reported to be diffusion-controlled, with sustained release for up to 3 – 8 weeks. In an alternative approach, drugs with active hydrogen groups (e.g., primary hydroxyl groups and amines), such as ascorbic acid¹⁰³, naphthalene compounds⁸⁸, and the anti-tumor drug 7-*tert*-butyldimethylsilyl-10-hydroxy-camptothecin (DB-67)¹⁰¹, were dissolved in the reactive PUR prior to injection and subsequently released in biologically active form by degradation of the polymer. Recent studies have reported that the release kinetics can be controlled by the composition of the drug⁸⁸, the choice of catalyst⁸⁷, or the addition of ionic ligands⁹⁹, which offer greater tunability compared to diffusion-controlled release. Potential advantages of covalently binding drugs to PUR scaffolds include the ability to increase drug loading and control release to achieve therapeutically relevant doses. For example, clinical evaluation of DB-67 has been limited by the inability to deliver it in sufficient quantities to slow tumor growth and disease progression. When DB-67 was covalently incorporated into LDI-glycerol PUR scaffolds, it was released *in vitro* at a rate suitable for delivering therapeutic concentrations of the drug and inhibited cellular proliferation by 50% for several malignant glioma cell lines^{101, 99}. While the degradation-controlled release mechanism has proven successful for small molecule synthetic drugs, another study has reported that recombinant human proteins dissolved in the aqueous phase prior to foaming are not released from the cured foam in a biologically active form⁹⁵.

Conclusion

The exponential growth in the development of minimally invasive surgical techniques has resulted in a compelling clinical need for injectable macroporous foams that enhance tissue healing. While there are a wide variety of technologies for producing macroporous implantable scaffolds, the use of harsh processing conditions, such as high temperatures and pressures and cytotoxic solvents, precludes the application of many technologies to the fabrication of injectable macroporous foams. In recent years, processes such as porogen leaching, gas foaming, and emulsion-templating have been modified for to generate

macroporosity in injectable biomaterials including calcium phosphates, hydrogels, and hydrophobic polymers. Recent developments reviewed in this manuscript are summarized in Table 2. Some of these technologies have shown promising results in preclinical and clinical studies and are progressing toward clinical translation.

In order to facilitate development of new injectable macroporous foam technology, a number of challenges need to be addressed. An additional phase, such as a solid degradable porogen, liquid-phase droplets in an emulsion, or gas bubbles that are generated within the material, must be utilized to create macropores within the scaffold after cure. The effects of removal of the additional phase from the scaffold must be considered in the design of injectable foams. Since the volume of the sacrificial phase must be relatively large and its rate of removal fast to create interconnected macropores, it can adversely affect surrounding host cells and tissue even if its toxicity is modest. Thus, the biocompatibility of solvents or oils used to fabricate polyHIPEs must be considered when these materials are injected *in situ*. Gas-blowing reactions present the challenge of volumetric expansion resulting from the creation of pores. While modest expansion can be helpful for filling voids and maintaining adequate contact between the material and the host tissue, too much expansion can complicate administration of the material since it may be difficult to estimate the appropriate injection volume. Other concerns that must be addressed in the design of injectable macroporous foams include the biocompatibility of catalysts and reactive intermediates prior to cure. Unlike implantable scaffolds, which can be washed extensively to remove undesirable impurities, all the components required to synthesize the foam must be injected into the defect, and thus every compound in the foam must be biocompatible and non-cytotoxic at the concentrations utilized. As future research addresses these critical areas of investigation, injectable macroporous foams are anticipated to have a significant impact on improving patient outcomes for a number of clinical procedures.

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Figure 1. Literature progression in the areas of minimally invasive surgery and injectable biomaterials. The plotted values correspond to the number of papers published each year related to these topics. The numbers were obtained by conducting a search in PubMed and including papers published up to 2012.

Figure 2.

SEM images of biphasic calcium phosphate cements. (A) A bi-phasic CPC fabricated by mixing α -tricalcium phosphate (α -TCP, 40%), calcium sulphate hemihydroate (CSH), and an aqueous solution containing 2.5 wt% Na_2HPO_4 . The cement initially contained 20 wt% $\alpha\beta$ -TCP ($\times 400$). Both small ($\sim 1 \mu\text{m}$) crystals of CSD (labelled D in Figure 2A and viewed at higher magnification in the inset image) and larger residual CSH (labelled H) crystals were observed in the cement. Voids formed by dissolution of the CSH crystals (labelled SH) are also evident in the SEM image.²⁹ (B) Microstructure of cements with 8.7 mM of SDS after 5 days of setting (L/P = 0.32 mL/g) shows macropores ($\sim 20 \mu\text{m}$) stabilized by the SDS³³.

Figure 3.

Immunostaining of cells remaining in macroporous fibrin scaffolds. Cells stained for myogenin, an early marker of muscle tissue differentiation after 8 days (A-C); cells stained for MYH after 16 days (D-F); and cells stained for ACTN and ACTA1 after 16 days of culture showing multi-cell merging indicating muscle fiber differentiation.

Figure 4. Shape memory cryo-gels as injectable materials. (A) Depiction of cryogel synthesis; (B) proof of injectability by loading a square shaped cryogel into a syringe and displaying the shape after injection; (C) rhodamine-labeled gels with varying size maintain their shape after injection; (D) variable geometries maintain their shape after injection; (E) stress vs. strain curves for conventional and cryogels showing recoverable deformation; and (F) SEM image of cell attachment to cryogels.

Figure 5. Images of macroporous and non-porous PPF based scaffolds. Scanning electron micrography (top) and magnetic resonance microscopy (bottom) images of either non-porous (a,e) or macroporous (b-d) PPF scaffolds.

Figure 6.
Schematic of the emulsion templating process to obtain a polyHIPE.

Figure 7.
Injectable propylene fumarate dimethacrylate (PFDMA) polyHIPE ⁴⁶.

Figure 8. Thermoresponsive injectable polyHIPEs ⁴⁷. (A) Diagram illustrating the formation of crosslinked oil-in-water thermoresponsive polyHIPEs using the temperature-triggered Lower Critical Solution Temperature (LCST) phase transition of polyNIPAAm chain segments as a means of non-covalently crosslinking injectable HIPEs (B) SEM image of a thermoresponsive dextran-b-polyNIPAAm polyHIPE with 20% (w/v) polymer concentration.

Figure 9.

Schematic summarizing the synthesis of injectable polyurethane (PUR) foams from viscous liquid precursors. Component 1 comprises a polyisocyanate or isocyanate (NCO)-terminated prepolymer. Component 2 includes all compounds with reactive hydrogen groups that can react with the prepolymer or control the properties of the foam, including the polyol, water, catalyst, matrix particles, and biologics. As evidenced by the rheological properties, at the $G' = G''$ crossover point (identified as the working time) the material transitions from a viscoelastic liquid to an elastic solid. Water reacts with the prepolymer to form carbon dioxide gas bubbles, which results in the formation of a macroporous foam (SEM image). In contrast to aromatic polyisocyanates, foams prepared from lysine-derived (LTI, LDI) or aliphatic (hexamethylene diisocyanate trimer (HDI)) show a mild ($<20^{\circ}\text{C}$) exotherm.

Figure 10.

Drug delivery from injectable PUR scaffolds. (A) Fluorescent images obtained using a DAPI filter depicting the distribution of naphthalene compounds in an LDI-glycerol PUR foam. Compounds were uniformly distributed throughout the foams, as evidenced by the blue color throughout the material. (B) SEM image of an LTI-polyester foam. (C) Confocal images of LTI-polyester foams augmented with FITC-labeled BSA as a labile powder. In contrast to the LDI-glycerol+naphthol foams, discrete BSA-FITC particles can be observed uniformly distributed throughout the material.

Table 1

Methods for characterization of injectable foams.

Type	Category	Parameter(s)	Method(s)
Handling	Viscosity	Shear rate profile, Injectability	Rheometer, Viscometer ⁴⁵ , MTS ^{38, 61}
	Cure profile	Working time (storage and loss modulus)	Rheometer, Gillmore needle ^{33, 38, 39}
Reaction conditions	Thermodynamics	Exotherm	DSC, Thermal couple ^{68, 69}
	Conversion	Composition	NMR ⁵ , FTIR ^{16, 34} , XRD ¹²
Static	Volumetric properties	Architecture: porosity, size, shape	SEM ^{5, 12, 28, 34, 38, 39, 45, 46, 57, 63, 68, 74} , mCT ⁵ , Stereological approach ⁶⁸ , Gravimetric ^{15, 33, 34, 38, 39, 46, 71, 74} , Mercury intrusion ³⁴
	Aqueous interaction	Swelling (volume), absorption (mass)	Gravimetric ^{5, 57, 68, 69}
Biocompatibility	Cellular interaction	<i>In vitro</i> : cell proliferation, viability, morphology	MTT ^{56, 59, 63} , live/dead ^{5, 17, 42, 46, 56, 57, 61-63} , SEM ¹⁷ , Microscopy ^{5, 56}
		<i>In vivo</i> : cell identification	Histology ^{16, 30, 42, 59}
Biomechanics	Rheology	Shear rate (Yield stress), Elastic modulus	Rheometer ⁵⁷
	Compression ¹⁵ , torsion, fatigue, tensile, three-point bending	Stress vs. strain, strength, modulus, energy to failure	DMA ⁷¹ , Instron ^{5, 12, 14, 46, 74} , MTS ^{28, 29, 33, 34, 38, 69} , Universal Testing Machine ⁶¹
Degradation	<i>In vitro</i>	Composition	Gravimetric ^{38, 39, 59} , HPLC
	<i>In vivo</i>	Presence	Histology ⁵⁹

Table 2

Summarized formulations of injectable macroporous foams for regenerative medicine and their intended applications.

Material(s)	Setting mechanism	Macroporous Formation	Intended Application
CPC: CDHA/CSD ²⁹	Reaction of α -TCP and CSH with water in an aqueous Na_2HPO_4 solution	CSD and CSH crystal dissolution	Bone void filler (CERAMENT™ spine and orthopaedic indications)
CPC: CDHA ³³	Reaction of α -TCP with water in an aqueous Na_2HPO_4 solution	Air entrainment using SDS	Hard tissue scaffold
CPC with polymeric porogens ³⁸⁻⁴⁰	Reaction of reactive calcium phosphate powders with water in an aqueous Na_2HPO_4 solution	Porogen (PLGA, poly(trimethyl carbonate, gelatin) leaching	Hard tissue scaffold
Hydrogel: Alginate/Fibrin ⁶³	Ionic/Enzymatic crosslinking	Mixing fibrin with degradable alginate/fibrin microbeads	Cell encapsulation material, soft tissue scaffold
Hydrogel: Methacrylated alginate/PEG ⁵	Free radical polymerization	Cryogelation	Cell delivery, Soft tissue scaffold
Fumarate: Poly(propylene fumarate) ⁷¹	Free radical polymerization	Mixing with degradable poly(DL-lactic-co-glycolic acid) microbeads	Soft or hard tissue scaffold
Fumarate: Oligo(poly(ethylene glycol) fumarate) ⁷²	Free radical polymerization	Mixing with degradable gelatin microparticles	Cell and drug delivery, Soft tissue scaffold
polyHIPE: PFDMA/PGPR ^{4125 46}	Radical cross-linking polymerization	Emulsion with an aqueous solution	Hard tissue scaffold
polyHIPE: Dextran-b-polyNIPAAm ⁴⁷	Thermo-responsive physical aggregation	Emulsion with p-xylene	Drug delivery, soft tissue scaffold
Polyurethane: LDI ^{78, 86} and LTI ⁸⁹ -derived prepolymers reacting with polyols	Reaction of isocyanate and alcohol groups to form urethane bonds	Blowing reaction of isocyanate with water to produce carbon dioxide gas	Soft tissue scaffold, drug delivery
Polyurethane: LDI ^{79, 93} and LTI ⁸¹ -derived prepolymers reacting with polyols	Reaction of isocyanate and alcohol groups to form urethane bonds	Addition of a matrix: DBM, CaP particles, allograft bone particles	Hard tissue scaffold, drug delivery

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