Bioactive composite materials for tissue engineering scaffolds

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Synthetic bioactive and bioresorbable composite materials are becoming increasingly important as scaffolds for tissue engineering. Next-generation biomaterials should combine bioactive and bioresorbable properties to activate *in vivo* mechanisms of tissue regeneration, stimulating the body to heal itself and leading to replacement of the scaffold by the regenerating tissue. Certain bioactive ceramics such as tricalcium phosphate and hydroxyapatite as well as bioactive glasses, such as 45S5 Bioglass®, react with physiologic fluids to form tenacious bonds with hard (and in some cases soft) tissue. However, these bioactive materials are relatively stiff, brittle and difficult to form into complex shapes. Conversely, synthetic bioresorbable polymers are easily fabricated into complex structures, yet they are too weak to meet the demands of surgery and the *in vivo* physiologic environment. Composites of tailored physical, biologic and mechanical properties as well as predictable degradation behavior can be produced combining bioresorbable polymers and bioactive inorganic phases. This review covers recent international research presenting the state-of-the-art development of these composite systems in terms of material constituents, fabrication technologies, structural and bioactive properties, as well as *in vitro* and *in vivo* characteristics for applications in tissue engineering and tissue regeneration. These materials may represent the effective optimal solution for tailored tissue engineering scaffolds, making tissue engineering a realistic clinical alternative in the near future.

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Relevance of bioactive synthetic scaffolds

The field of tissue engineering provides an alternative approach to existing treatment strategies for the repair and regeneration of damaged human tissue. Organ and tissue transplants are imperfect solutions, due to lack of donor tissue and possible donor site morbidity [1]. Tissue engineering draws on principles from biology and engineering science for the development of functional substitute materials to regenerate living tissue, where loss or damage has occurred due to injury or disease [2]. Moreover, the development of strategies for *in situ* tissue regeneration and repair presents clinical and economic advantages, especially regarding care for an aging population [3,4]. The scientific challenge in tissue engineering encompasses understanding the cells themselves, their mass transport requirements and physiologic environment, in conjunction with the development of optimal scaffold materials that are usually porous and biodegradable and act as temporary 3D templates for cell adhesion, proliferation, migration and ultimately, the formation of new tissue [5]. The structure and properties of these scaffolds are pertinent to the tissue concerned and the loads it will experience *in vivo* [6]. The generic requirements for ideal tissue engineering scaffolds are listed in BOX 1 [5–7].

Synthetic biodegradable polymers are the most widely considered materials for the development of tissue engineering scaffolds. In particular, polyesters such as polylactic acid (PLA), polyglycolic acid (PGA) and their copolymers polylactid-co-glycolic acid (PLGA) [8–10]. PGA, PLA, polydioxane and

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their copolymers are the only synthetic and biodegradable polymers with extensive USA Food and Drug Administration (FDA) approval history. Numerous other bioresorbable materials are being suggested for use in scaffolds, including naturally occurring [11,12] and totally synthetic polymers [13], bioactive porous ceramics produced by foaming methods such as sol-gel [14], rapid prototyping [15] and replicating techniques [16], as well as vital–avital composites, that is, composites made of living and nonliving matter [17]. Moreover, composites based on biodegradable polymer matrices with the addition of inorganic bioactive phases such as hydroxyapatite (HA) or bioactive glass, in the form of particles or fibers, are increasingly being considered for use as bone tissue engineering scaffolds due to their improved physical, biologic and mechanical properties, and in particular the capacity they offer in tailoring their structure and degradation rate to the specific need at the implant site [18–21]. Much current research is focused on the development of a great variety of such bioactive and biodegradable composite materials, as both solid and porous systems with the bioactive phase incorporated either as filler or coating (or both) into the biodegradable polymer matrix (FIGURE 1). Until recently, most research was based on composites incorporating HA or calcium phosphate particulates in biodegradable polymeric systems, and some typical examples of these composites are presented in references [22–25]. This review focuses on the state-of-the-art development of biodegradable and bioactive composite scaffolds based on synthetic polymers and bioactive glasses, in terms of their fabrication processes, microstructure characterization, mechanical properties, as well as, *in vitro* and *in vivo* characteristics for their application in both hard- and soft-tissue engineering. The composites to be considered here are developed purely from synthetic materials, thus systems which include biologic compounds, that is, biomolecules or growth factors in their structure are not covered.

Box 1. Requirements for tissue engineering scaffolds [5,7].

- Suitable biocompatibility with predictable biodegradation that is matched to the invasion of neotissue
- High porosity, of a 3D nature, ideally possessing an interconnected pore network to facilitate the migration of cells and permit fluid flow for nutrient supply and the removal of cellular waste products, and to permit vascular invasion
- Suitable bioactivity to exploit the body's natural repair process – by influencing the genes in the generating cells to enable efficient cell differentiation and proliferation
- Optimal surface chemistry for cell attachment, proliferation and differentiation
- Sufficient mechanical integrity
- Easy to process into 3D complex shapes in a controllable and reproducible manner

Characteristics of biomaterials used

Bioactive glasses & ceramics

Bioactive ceramics, such as HA, tricalcium phosphate (TCP) and certain compositions of silicate and phosphate glasses (bioactive glasses) and glass ceramics (e.g., apatite–wollastonite) react with physiologic fluids and through cellular activity to form tenacious bonds to hard (and in some cases soft) tissue [26]. A characteristic of many bioactive materials is the formation of a biologically active HA layer in the presence of body fluids *in vitro* or *in vivo*. The materials with the highest levels of bioactivity develop a silica gel layer that promotes HA formation [26,27]. Thus, HA formation on material surfaces upon immersion in acellular simulated body fluid (SBF) is considered a qualitative measure of bioactivity. Bioactive glasses (e.g., 45S5 Bioglass®) with compositions in the system SiO_2-Na_2O-CaO-P_2O_5, having less than 55% SiO_2, exhibit high bioactivity index (class A), and bond to both soft and hard connective tissues [26]. The class of bioactivity depends on the rate and type of tissue response. Class A bioactive materials are osteoproductive (bone grows on material surfaces due to enhanced bonding along a surface). Class B bioactive materials such as HA exhibit osteoconductivity only. 45S5 Bioglass® is used in the clinic as a treatment for periodontal disease (Perioglas®) and as a bone filling material (Novabone®) [27]. Bioglass® implants have also been used to replace damaged middle ear bones, restoring the hearing to thousands of patients [27].

It has recently been found that reactions on bioactive glass surfaces release critical concentrations of soluble Si, Ca, P and Na ions, which induce intra- and extracellular responses [28]. For example, a synchronized sequence of genes is activated in osteoblasts that undergo cell division and synthesise an extracellular matrix, which mineralizes to become bone [28]. In addition, bioactive glass compositions doped with AgO_2 have been shown to elicit antibactericidal properties while maintaining their bioactive function [29]. In recent investigations, 45S5 Bioglass® has also been shown to increase secretion of vascular endothelial growth factor (VEGF) *in vitro* and to enhance vascularization *in vivo*, suggesting scaffolds containing controlled concentrations of Bioglass® may stimulate neovascularization, which is beneficial to large tissue engineered constructs [30].

The sol-gel process is an alternative method to produce bioactive glasses [27]. An advantage of gel-derived over melt-derived bioactive glasses is that they exhibit a mesoporous texture (pores with diameters in the range of 2–50 nm). This texture enhances bioactivity and resorbability of the glasses [31]. The excellent properties of bioactive glasses and their long history of applications in biomedical implants has recently prompted extensive research regarding their use in tissue engineering and regeneration strategies [25], mainly in the form of powder aggregates, porous substrates (foam scaffolds) [32–35], and also as a particulate addition – as filler or coating to biodegradable polymers, which is the focus of this review.

Resorbable polymers

The most widely used biodegradable polymers in tissue engineering are aliphatic polyesters, such as PLA, PGA, polycaprolactone (PCL), polyethylene oxide (PEO), poly(3-hydroxy-

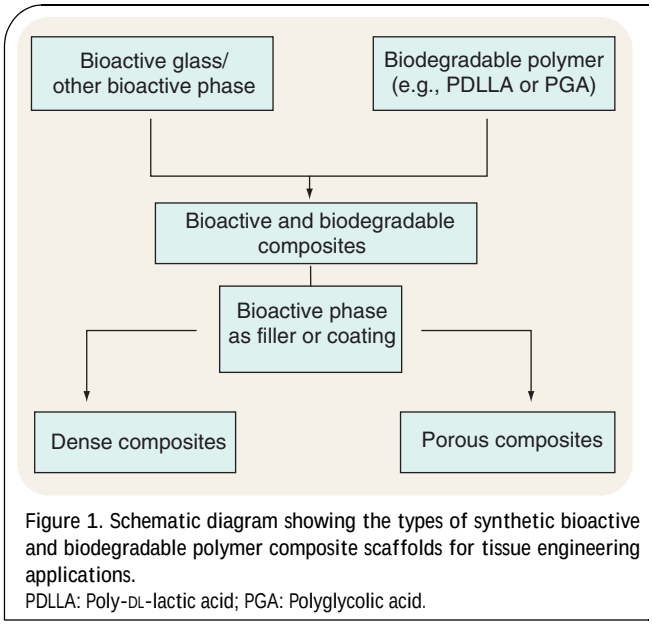

Figure 1. Schematic diagram showing the types of synthetic bioactive and biodegradable polymer composite scaffolds for tissue engineering applications.
PDLLA: Poly-DL-lactic acid; PGA: Polyglycolic acid.

butytrate) (PHB) and blends thereof [8–10,36]. Possible risks such as toxicity, immunogenicity and favoring of infections are lower for pure synthetic polymers with constituent monomeric units having a well-known and simple structure. Poly(α-hydroxyesters) PLA, PGA and PCL have demonstrated clinical success as surgical sutures, meshes and drug delivery systems [13]. Degradation of these polymers occurs by chemical hydrolysis and they are insensitive to enzymatic processes, therefore degradation does not vary from patient to patient [9]. Moreover, PLA, PGA, polydioxane and their copolymers are the only synthetic and biodegradable polymers with an extensive FDA approval history. These polymers can be fabricated as sponge-like sheets, gels or complex structures with intricate porous networks and channels using conventional polymer processing techniques, as reviewed in the literature [37]. Biodegradable polyester degradation occurs by uptake of water followed by hydrolysis of ester bonds. Many different factors affect the degradation kinetics, such as chemical composition and configurational structure, processing history, molar mass (Mw), polydispersity (Mw/Mn), environmental conditions, crystallinity, device size, morphology (e.g., porosity) and chain orientation, distribution of chemically reactive compounds within the matrix, additives [38,39], presence of original monomers and oligomers, and overall hydrophilicity. Thick samples of these polymers can lead to heterogeneous degradation, faster inside than at the exterior [40]. Heterogeneous degradation may be ascribed to two phenomena:

- Easier diffusion of soluble oligomers from the surface into the external medium than from inside
- Neutralization of carboxylic end-groups located at the surface by the external buffer solution (*in vitro* or *in vivo*)

These contribute to reduce the acidity at the surface, whereas in the bulk, degradation rate is enhanced by autocatalysis due to carboxylic end groups. Indeed, hydrolysis of amorphous polymers, for example PDLLA, is faster due to the lack of crystalline regions and thus enhanced water uptake. In general, the amount of absorbed water depends on diffusion coefficients of chain fragments within the polymer matrix, temperature, buffering capacity, pH, ionic strength, additions in the matrix, additions in the medium and processing history. Different aliphatic polyesters thus exhibit very different degradation kinetics in aqueous solutions. PGA, for example, is a stronger acid and behaves more hydrophilically than PLA, which is hydrophobic due to its methyl groups. The stereochemistry (the conformation each unit takes in space with respect to others) influences the final properties; better alignment of neighbors leads to higher crystallinity. Degradation takes longer with the stereoisomers of the polymer such as, PLA composed of L-lactic repeating units takes more than 5 years for total absorption, whereas approximately 1 year is needed for amorphous PLA (or PDLLA) [41] and PCL can take several years to degrade *in vivo* [6,42]. PLGA has a wide range of degradation rates, the degradation kinetics being governed by both hydrophobic/hydrophilic balance and crystallinity. Composition of chains (i.e., contents in L-LA and D-LA and/or GA units) determines the degradation rate of PLGA polymers. Blends containing the greatest amount of PGA have been demonstrated to degrade faster [43]. In general, the initial degree of crystallinity of polyesters affects the rate of hydrolytic degradation because the crystal segments reduce water permeation in the matrix. Of particular significance for applications in tissue engineering, debris and crystalline byproducts [44], as well as acid degradation products of PLA, PGA, PCL and their copolymers, have been implicated in adverse tissue reactions [6,45]. The degradation of polymers in the presence of basic compounds depends on base catalysis, the neutralization of carboxyl end groups and the amount and morphology of incorporated compounds. Several groups have incorporated basic compounds to stabilize the pH of the environment surrounding the polymer and to control its degradation. Bioactive glasses, or more commonly calcium phosphate ceramics, have been used [42–44]. In particular, the addition of 45S5 Bioglass® has been shown to increase water absorption compared with pure polymer foams of PDLLA [20] and PLGA [21,46]. The possibility of altering the degradation kinetics of polyesters by addition of inorganic phases is thus another reason behind the development of polymer/bioactive glass composites.

Composite materials strategy

The brittleness of ceramics put them at a disadvantage as scaffold materials. Polymers are easily fabricated to form complex shapes and structures yet, in general, they lack a bioactive function (e.g., strong bonding to living tissue) and are too flexible and weak to meet the mechanical demands in surgery and in the physiologic environment. There are thus several reasons for the combination of biodegradable polymers and bioactive ceramics or glasses for tissue engineering, and other biomedical applications. First, the combination of polymers and inorganic phases leads to composite materials with improved mechanical properties, exploiting the well known composites approach by incorporation of stiffer particles into the softer matrix [47]. Moreover, as

mentioned above, addition of bioactive phases to bioresorbable polymers can alter the polymer degradation behavior, by allowing rapid exchange of protons in water for alkali in the glass or ceramic. This mechanism provides a pH buffering effect at the polymer surface, so acceleration of acidic degradation of the polymer can be controlled [21]. Filler materials have been shown to influence the degradation mechanism by preventing the autocatalytic effect of the acidic end groups resulting from hydrolysis of the polymer chains. Thus, composites can exhibit an erosion type of degradation rather than bulk degradation [39]. Development of composite materials is also attractive since their properties can be engineered to suit the mechanical and physiologic demands of the host tissue by controlling the structure of the polymer and volume fraction, and arrangement of the reinforcing phase [7,17]. Recently, inclusion of bioactive glasses has been shown to modify surface and bulk properties of composite scaffolds by increasing the hydrophilicity and water absorption of the hydrophobic polymer matrix [48,201], thus altering the scaffold degradation kinetics [51,201]. In addition, composites are able to absorb water due to the internal interfaces formed between the polymer and more hydrophilic bioactive phases. It has been reported that polymer composites filled with HA particles hydrolyzed homogeneously due to water penetrating the interfacial regions [52]. In general, the degradation and resorption kinetics of composite scaffolds are designed to allow cells to proliferate and secrete their own extracellular matrix while the scaffolds gradually vanish, leaving space for new cell and tissue growth. The physical support provided by the 3D scaffold should therefore be maintained until the engineered tissue has sufficient mechanical integrity to support itself.

The last, but not least important reason driving the development of polymer/bioactive glass composite scaffolds for tissue engineering is the need for conferring bioactive behavior to the base polymers, which is achieved by the bioactive fillers (inclusions) or coatings, as mentioned previously. Bioactivity, as determined by the rate of ion release and calcium phosphate formation, can be controlled by the volume fraction, size and shape of inclusions [7,22–25,53]. It has been shown that increased volume fraction and higher surface area to volume ratio of inclusions favor higher bioactivity, hence in some applications the incorporation of fibers instead of particles is favored [54,55]. In general, the bioactive and degradable composites reviewed here can be classified into dense or porous, which can be filled or coated with the bioactive phase in the form of particulates or fibers, as shown schematically in FIGURE 1. A summary of relevant mechanical properties of constituent phases most widely used in the production of bioactive and biodegradable composite scaffolds is shown in TABLE 1.

Clinically relevant tissues under investigation include musculoskeletal (bone, cartilage and muscle), epidermis, liver, kidney, lung, cardiovascular and nervous tissues, urothelum, bladder and endocrine pancreas [56,57]. However, the development of polymer-bioactive glass composite scaffolds has mainly focused on bone and cartilage tissue engineering. Only limited work has been performed on developing composite scaffolds for other tissues such as the intestine [58] and lung tissue [59]. As mentioned above, the requirements of scaffolds for tissue engineering are complex and specific to the structure and function of the host tissue and defect type. Moreover, the rate of tissue remodelling depends on the anatomy and physiology of the host tissue, therefore, bioactivity, degradation, porous architecture and time-dependant mechanical properties must be tailored for each case and no universal designing parameters are available. For example, the cell diameter in suspension dictates the minimum pore size required. For bone tissue engineering pore sizes 40–100 µm are necessary for osteoid ingrowth but this value might not be correct for other cell types [60]. The scaffold must also maintain differentiated functions without hindering proliferation. In regenerating tissues in volumes greater than a few mm^3, a capillary network becomes necessary for gas exchange, provision of nutrients and elimination of waste products for the survival of a large mass of cells. These functions usually necessitate the use of optimal bioreactors [57]. Indeed, depending on the size of the defect, the matrix may require seeding with exogenous cells and cultured *in vitro* prior to implantation. Moreover, there is a trade off between mechanical properties and porosity. As opposed to load-bearing dense structures, a highly porous scaffold represents a temporary mechanical support for the cells, possessing sufficient mechanical integrity to support itself in early development, to enable surgical application and manipulation, and to resist the forces of wound contraction without damage to the pore structure. Such highly porous composite scaffolds as discussed in this review are most appropriate for application as bone-filling materials for critically sized defects. Bone properties vary from anatomical site and there are well known density and structural organization differences between cortical and trabecular bone. Although the pore structure of polymer/ceramic composite scaffolds can be tailored to the implant site, their poor mechanical properties make them unsuitable for load bearing applications in general, and additional mechanical support must be provided. Tissues such as muscle, tendon, ligaments, blood vessels, nerves, bone and teeth have tubular or fibrous bundle architectures with anisotropic properties and therefore, scaffold architectures with orientated porosity, formed by suitable fabrication techniques are required, as discussed below.

Fabrication & properties of bioactive & biodegradable composite scaffolds

Dense bioactive glass-filled composites

Dense and hard composites of high strength and good processability have applications in orthopedic, oral, maxillofacial and craniofacial surgery as screws, pins, plates and bone fixation devices. This important group of hard tissue augmentation devices differs from tissue engineering scaffolds, as they do not permit the rapid invasion of cells and tissue formation in 3D, with the exception of those which have rapidly resorbing phases or contain *in situ* porosifiers. Dense bioactive glass containing biodegradable polymers will therefore be only briefly considered

Table 1. Mechanical properties of human bone, bioactive phases and synthetic degradable polymers.

Substance	Elastic modulus (GPa)	Strength (MPa)	Elongation (%)	Ref.
Cortical bone (human femur, tested dry in compression)	14.7–19.7	167–215		[81]
Cancellous bone (human distal femur, tested dry in compression)	0.298 ± 0. 224	5.6 ± 3.8		[82]
Bioglass®	35	42		[26]
HA	95	50		[26]
PDLLA	1.4–2.8	27.6–41.4	3–10	[6]
PLGA	1.4–2.08	41.4–55.2	3–10	[6]
PLLA	2.4–4.2	55.2–82.7	5–10	[6]
PGA	>6.9	>68.9	15–20	[6]
PCL	0.21–0.34	20.7–34.5	300–500	[6]

HA: Hydroxyapatite; PCL: Polycaprolactone; PDLLA: DL-polylactic acid; PGA: Polyglycolic-acid; PLGA: Polylactid-co-glycolic-acid; PLLA: Poly(lactic acid).

here for completeness, and mainly regarding devices for bone filling or augmentation. Many groups have developed augmentation devices using bioactive glasses [42,54,55,61] or HA [52] in polyester matrices using blending, extrusion, compounding and compression moulding, even exploiting the forging process to induce crystal (multiaxial) orientation, which resulted in matrix self reinforcement. Self-reinforced PL/DLLA (70:30) matrix composites containing bioactive glass (BG13–93) have been prepared by Niiranen and colleagues [49]. Addition of bioactive glass was found to modify the degradation kinetics *in vitro* and *in vivo* and the material morphology. Moreover, addition of bioactive glass was beneficial in terms of dimensional stability, neutral degradation and apatite formation on composite surfaces. Significant swelling of the neat polymer samples was found to occur (50–60% at 24 weeks), whereas the composites retained their diameter after 75 weeks of *in vitro* degradation. Bioactive glass addition usually increases the hydrophilicity of composites by the presence of the glass/matrix interfaces. Chan and colleagues prepared Bioglass®/Dextran® composites to overcome the lack of cohesiveness of particulate Bioglass® (grade 45S5) when used in augmenting bony surfaces [62]. Addition of medium molecular weight Dextran (a polysaccharide) modified the particulate to a putty consistency and improved handling characteristics. No adverse tissue reaction was observed and Dextran was removed from the site within the first week after implantation. Such composites are able to be formed in the implantation site during surgery and may be suited to areas where functional demand is placed on the graft site. Recently, an injectable composite of particulate bioactive glass ($S_{53}P_4$) and poly(ε-caprolactone-co-D,L-lactide) as a thermoplastic carrier matrix has been developed [63]. This material is interesting as bone filler in cancellous and cartilaginous subchondral bone defects. The copolymer proportions were adjusted to ensure sufficient flow properties for injection at 47–50°C. Studies have also been performed using sucrose particles as *in situ* porosifiers in a composite containing 50 wt% bioactive glass and 20 wt% sucrose in a copolymer matrix, however; the length of the study (23 weeks) may not have been enough to notice significant bone ingrowth [63]. No effective merit in incorporating the *in situ* porosifier was reported. It is possible that the porosifier loading was too small and did not act effectively. Additionally, the chosen polymer (96PCL:4PDLLA) may not resorb rapidly enough *in vivo* [42]. It is likely that more research efforts will be extended into these *in situ* porosifier containing composite materials, especially with regard to filling bone defects by injection and shaping the material during surgery. On the borderline of the composite/nanocomposite systems being considered in this review, it is worthwhile mentioning recent work on bioactive and biodegradable organic polymer–inorganic hybrids based on PCL and SiO_2, which are produced by sol-gel methods [48,64–67]. Silica (or titania) precursors are polymerized with a flexible polymer at molecular level to form an inorganic–organic hybrid. These hybrids have demonstrated bioactivity depending on the amount of silica (or titania) present. Silanol groups which exist in the silica phase of a bioactive organic/silica hybrid are known to provide nucleation sites for the formation of apatite crystals. Crystalline HA formation has been reported to occur within 7 days of immersion in SBF [65]. An increase in the polymer content has been shown to reduce bioactivity in these systems, however, the mechanical properties can be altered between hard-brittle (ceramic like) to ductile-tough (polymer like) behavior [48]. Mechanical properties reported for these systems are in the ranges of 0.06 to 0.3 MPa and 5 to 20 MPa for elastic modulus and tensile strength, respectively, with strain to failure in the range of 20 to 250% (in tension) depending on polymer content [48,67]. Moreover, the surface properties can be tailored by adjusting the PCL to silica content, as determined from wettability tests using contact angle measurement [48]. This effect has also been found in 45S5 Bioglass® reinforced PDLLA [201]. The authors are not aware of any porous

versions of these hybrid materials, indeed the use of PCL would suggest these materials have very long resorption times and may not be suitable for tissue engineering until sufficient porosity is introduced by foaming, possibly adapting processes developed for sol-gel derived bioactive glasses [68].

Dense bioactive glass-coated composites

Pressing and slurry dipping techniques have been used to coat commercially available surgical sutures and meshes with bioactive glass particles [69,70]. Stamboulis and colleagues prepared composites of polyglactin 910 (Vicryl®) sutures coated by 45S5 Bioglass® particles with an average particle size of less than 5 µm using a simple layer pressing procedure [71]. Sutures were pressed onto Bioglass® powder using flat surface steel plates. After 28 days of *in vitro* incubation in SBF, the tensile strength of the coated sutures was higher, indicating the protective function of the Bioglass® coating [71]. Both the extent and rate of suture degradation were reduced due to a pH buffering effect at the polymer surface. The group of Tormala and colleagues has been particularly active in the development of bioactive glass coated degradable polymer devices [72,73]. Mechanical and bioactive properties of dense composites of PLA pressed with bioactive glasses have been shown to suit applications in cranial, maxillofacial and guided bone regeneration approaches [72,73].

The coating of biodegradable polymer substrates with inorganic bioactive particles has also been investigated as part of biomimetic strategies [64,74–76]. In these approaches, calcium phosphate coatings which are similar to bone apatite, are produced *in situ* upon immersion of the substrates in relevant solutions with tailored ion concentrations. These materials, however, do not rely on the presence of a bioactive phase coating, such as Bioglass®, to induce the calcium phosphate deposition.

Porous bioactive glass-filled composites

Porous composites of bioactive glass-containing polymers have been prepared by numerous different techniques, including thermally induced phase separation (TIPS), compression moulding and particulate leaching, gas foaming and sintering of composite microspheres [7,19–21,46,50,77–79,301]. The most popular processing techniques developed for the production of highly porous scaffolds, including their relative advantages and disadvantages are summarized in TABLE 2. The main techniques are discussed in the following paragraphs.

Thermally induced phase separation

The 3D resorbable neat polymer foams with very high porosity (~97%) are commonly produced using the TIPS technique to give controlled macro- and microstructures suitable as scaffolds for tissues such as nerve, muscle, tendon, ligament, bone and teeth [8,87]. Only a few research groups, however, have been active in producing bioactive glass containing polyester matrix composites using the TIPS method [19–21]. A schematic diagram describing the TIPS process for fabrication of polymer/Bioglass® composite foam scaffolds is presented in FIGURE 2. These scaffolds are highly porous with anisotropic tubular morphology and extensive pore interconnectivity. Microporosity of TIPS-produced foams, their pore morphology, mechanical properties, bioactivity and degradation rate can be controlled by varying polymer concentration in solution, volume fraction of the reinforcing phase as well as quenching the temperature and the polymer and solvent used [46]. TIPS can be considered the processing method of choice if scaffolds with highly oriented porosity need to be fabricated. Maquet and colleagues [20,21], for example, have used an optimized TIPS process to develop highly porous poly(D,L-lactide)/Bioglass® composite scaffolds exhibiting bimodal and anisotropic pore structures composed of tubular macropores of approximately 100 mm in diameter, interconnected with a micropore network of approximately 10–50 µm in diameter, as shown in FIGURES 3A–C [46]. This pore structure differs considerably from that achieved by the traditional particulate salt leaching process, in this case, foams

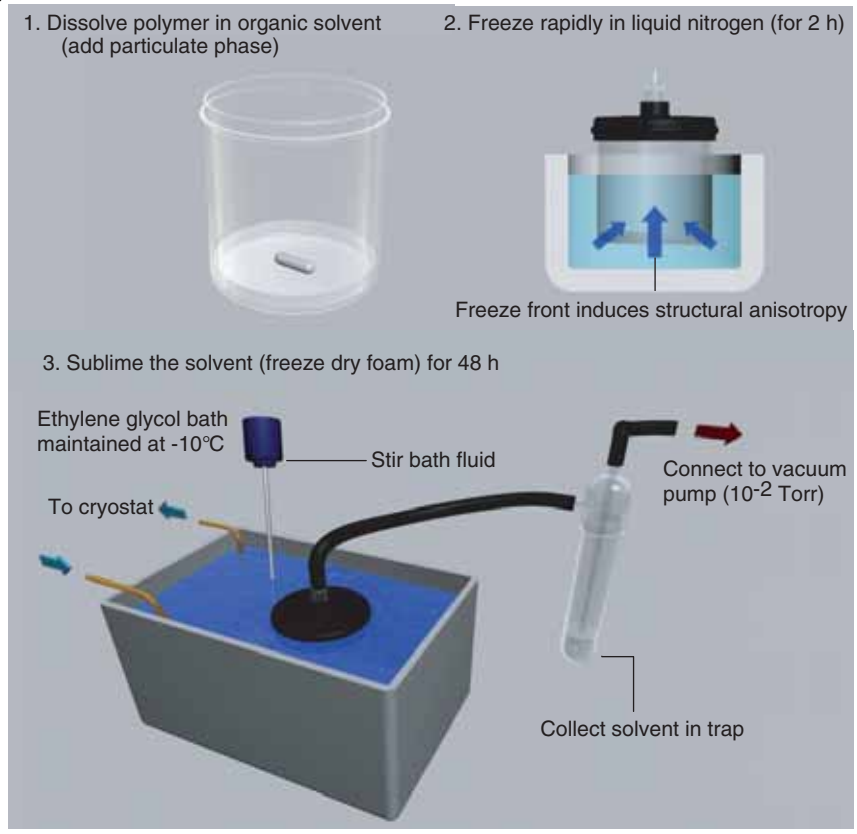

Figure 2. Schematic representation of the thermally induced phase seperation process for fabrication of composite foam scaffolds.

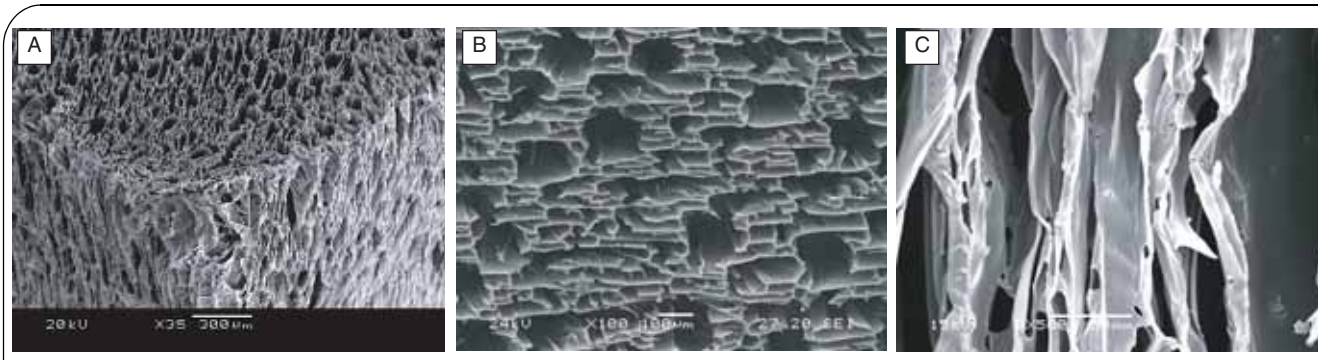

Figure 3. Scanning electron micrographs of a Bioglass®-filled DL-polylactic acid foam showing (A) macrostructure of the highly ordered porosity, (B) bimod pore size distribution and (C) regular arrangement of the tubular macropores demonstrating pore anisotropy [20,21].

exhibit a more isotropic structure with equiaxed pores but much less interconnectivity, as the micrograph of FIGURE 4 demonstrates. In TIPS-produced foams, the pore volume has been shown to decrease from 9.5 to 5.7 cm^3/g with addition of 40 wt% Bioglass®, with little change observed in the overall pore morphology. *In vitro* studies in phosphate buffered saline (PBS) at 37°C showed that the addition of Bioglass® increased water absorption and weight loss in comparison with pure polymer foams [20,21]. Molecular weight was found to decrease less in the composite foams possibly due to the dissolution of alkaline ions from the Bioglass® providing a pH buffering effect, as discussed above. Both the PDLLA/Bioglass® composites and neat PDLLA foams retained their structural integrity until the end of the experiment (16 weeks), which means degradation was still in the early stages [21]. An extended study (up to 6 months) should improve the understanding of the degradation kinetics of these composite scaffolds. Indeed, it is well documented that due to autocatalysis, nonporous materials undergo degradation more rapidly than porous materials as porous materials are able to facilitate dissolving and spreading of degradation products throughout the aqueous medium, thereby preventing autocatalysis [46,87,88]. PDLLA/Bioglass® composites exhibit high bioactivity, assessed by the formation of HA on the composite surfaces upon immersion in SBF, as shown in FIGURE 5. It has also been shown that the foams support the migration, adhesion, spreading and viability of MG-63 cells (osteosarcoma cell line), a representative scanning electron microscopy (SEM) micrograph documenting these findings is shown in FIGURE 6 [89]. The potential of these scaffolds in bone and soft-tissue engineering has been demonstrated *in vitro* with optimal concentrations of 45S5 Bioglass® added to PDLLA or PLGA matrices [89,90]. Highly porous tubular scaffolds with oriented porosity have also been fabricated by exploiting the TIPS process [58,90]. These are candidate materials for soft-tissue engineering with potential application in the regeneration of tissues requiring tubular shapes such as the intestine, trachea and blood vessels. TIPS fabricated PDLLA foams with and without Bioglass® additions have been shown to exhibit mechanical anisotropy concomitant with the TIPS induced pore architecture [91]. For comparison, the mechanical properties of a selection of highly porous scaffolds produced by different methods are shown in TABLE 3. Inclusion of stiff inorganic bioactive phases gives slight improvement in the Young's modulus and compression strength of scaffolds. Polymer matrix composite films containing nano-size titania and other inorganic particulate inclusions have demonstrated enhanced cell adhesion and propensity to increased calcium-containing mineral deposition [92]. Recently, 3D PDLLA foams containing both TiO_2 nanoparticles and Bioglass® additions have been synthesized by TIPS, which demonstrate enhanced bioactivity and surface nanotopography [93].

Rapid prototyping

Solid freeform fabrication techniques, such as fused deposition modelling (FDM), have been also employed to fabricate highly reproducible scaffolds with fully interconnected porous networks [5,85]. Using digital data produced by an imaging source such as computer tomography (CT) or magnetic resonance imaging (MRI), enables accurate design of the scaffold structure [5]. Solid free form (SFF) manufacturing coupled with conventional foam scaffold fabrication procedures (phase separation, emulsion-solvent diffusion or porogen

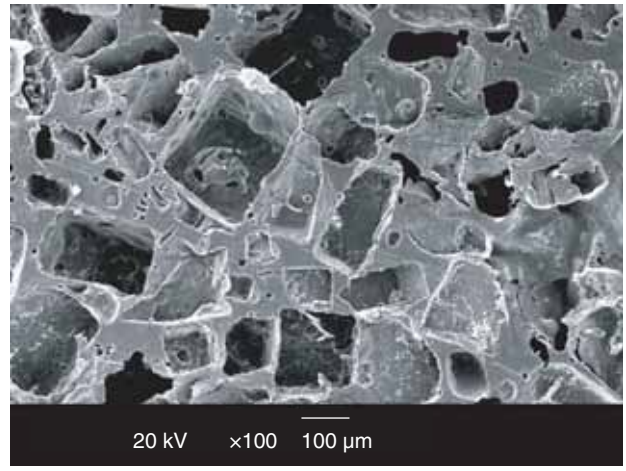

Figure 4. Scanning electron micrograph of a porous scaffold obtained by the salt particle leaching process showing limited pore interconnectivity and isotropic pore structure with almost equiaxed pores.

Table 2. Fabrication routes for highly porous scaffolds, their advantages and disadvantages.

Fabrication route	Advantages	Disadvantages	Ref.
TIPS	High porosities (~95%)	Long time to sublime solvent (48 h)	[8,20,83]
	Highly interconnected pore structures	Shrinkage issues	
	Anisotropic and tubular pores possible	Small scale production	
	Control of structure and pore size by varying preparation conditions	Use of organic solvents	
Compression moulded/particulate leaching	Controlled porosity	Poor interconnectivity especially at low porosities	[18,62]
	Graded porosity structures possible	Difficult to generate large structures (>3 mm thick)	
	No organic solvents	Not all particulates leached	
		Heat may damage encapsulations	
Solvent casting/particulate leaching	Controlled porosity	Structures generally isotropic	[84]
	Controlled interconnectivity (if particulates are sintered)	Use of organic solvents	
Microsphere sintering	Graded porosity structures possible	Interconnectivity is an issue	[77]
	Controlled porosity	Use of organic solvents	
	Can be fabricated into complex shapes		
Solid freeform	Porous structure can be tailored to host tissue	Resolution needs to be improved to the microscale	[85,86]
	Protein and cell encapsulation possible	Some methods use organic solvents	
	Good interface with medical imaging		

TIPS: Thermally induced phase separation

leaching) may be used to develop scaffolds with controlled locally porous and globally porous internal architectures. Such biomimetic internal architectures may prove valuable for multi-tissue and structural tissue interface engineering. However, to the authors' knowledge, there is no literature available on bioactive glass/degradable polymer composites made by solid free-form fabrication techniques, this technique has been only applied for composites containing calcium phosphates as the bioactive phase [85,86]. For example, Xiong and colleagues fabricated composites of PLLA/TCP with porosities of up to 90% and mechanical properties close to human cancellous bone by using low-temperature deposition based on a layer-by-layer manufacturing method of solid freeform fabrication (computer-driven by 3D digital models) [86]. PLLA was dissolved in dioxane and TCP powder mixed to prepare a slurry, which was formed into frozen scaffolds, and subsequently freeze dried. Alternate parallel layers formed macropores (400 µm diameter) and sublimation of the solvent during freeze drying formed micropores (5 µm diameter). Taboas and colleagues produced PLA scaffolds with computationally designed pores (500–800 µm wide channels) and solvent fashioned local pores (50–100 µm wide voids or 5–10 µm length plates) [85]. Indirect fabrication using casting in SFF moulds provided enhanced control over scaffold shape, material, porosity and pore architecture, including size, geometry, orientation, branching and interconnectivity. A shortcoming of this route is increased scaffold fabrication time compared with direct methods, as a temporary mould must be made. However the casting operation may improve the thickness weakness inherent in layer-by-layer manufacturing.

Rapid prototyping techniques offer control over the matrix architecture and may be integrated with imaging techniques to produce tissue or patient specific scaffolds [85]. Mouldless fabrication and rapid prototypting techniques which also enable 3D encapsulation of cells and SFF techniques are likely to become more effective as they approach micron-scale resolution [94]. Such techniques, when avoiding solvents and high temperatures are advantageous for protein and cell encapsulation.

Other processing routes

After the first investigations on highly porous 3D scaffolds made of bioactive glass filled PDLLA and PLGA, published in 2002 [7], an increasing number of publications are beginning to emerge on this subject, as discussed next. 3D composites of bioactive glass and degradable polymers have been produced by sintering, for example, composite microspheres by Lu and

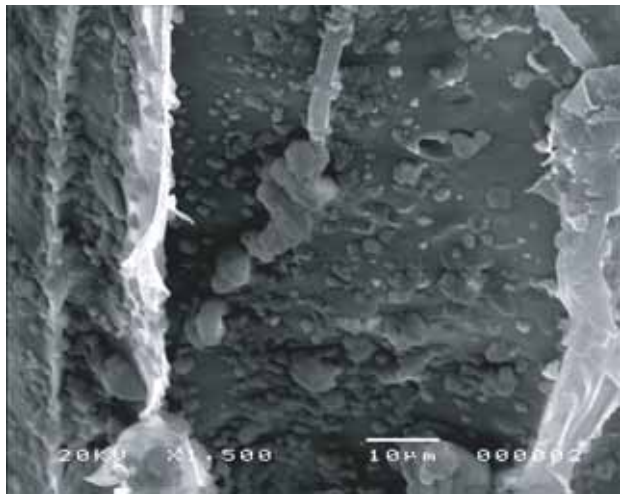

Figure 5. Scanning electron micrograph image showing the surface of a 5 wt% Bioglass® filled DL-polylactic acid foam after 21 days immersion in simulated body fluid. Demonstrating the formation of HA crystals on the polymer surface, which is an indication of the highly bioactive nature of these foams.

colleagues [77]. Starting materials were PLAGA-Bioglass® composite microspheres obtained through a water–oil–water emulsion technique. Sintering of the microspheres into cylindrical shapes resulted in a well-integrated interconnected porous structure, with the microspheres joined at the contact necks. The average porosity was 40% with pore diameters of 90 µm, and the mechanical properties were close to cancellous bone. The composites were shown to be bioactive as a calcium phosphate layer formed on the surface of the composite on immersion in SBF for 7 days. Moreover, Bioglass® reinforcement gave a twofold increase in compressive strength. The scaffolds were shown to support the adhesion, growth and mineralization of human osteoblast-like cells *in vitro*. Over a 3-week period, cultures with PLAGA/Bioglass® maintained pH variations within physiologic ranges. More recently, Yao and colleagues synthesized PLGA/bioactive glass microspheres by emulsification and heated them in moulds to fabricate porous 3D scaffolds [78]. They demonstrated the bioactivity of the composites and their ability to promote osteogenesis of marrow stromal cells. In related research, CO_2 gas-foaming procedures have been used to create silica/PDLLA composites [53]. However, the authors reported relatively low porosities (40–55%). There has been little work performed on producing bioactive glass-polymer scaffolds using particulate leaching. A problem with this technique is achieving pore interconnectivity at low porogen (salt/sucrose) loadings, as many of the porogen particles may remain trapped (FIGURE 4). Nevertheless, composites based on calcium phosphate inclusions with variable and graded porosity have been produced using this route [95]. Such composites can be fine tuned to meet the specific requirements of the host site. Related biodegradable composites containing silicate phases other than 45S5 Bioglass® in biodegradable polymer matrices have been investigated recently, including PLA with soluble calcium phosphate particles [96], and PDLLA with wollastonite [97]. Alternative matrices based on natural polymers, (e.g., starch-based polymers), have also been investigated for biodegradable polymer/Bioglass® composites [98].

Porous bioactive glass-coated composites

Porous Bioglass® coated scaffolds, either as foams [99], fibrous bodies [100] or meshes [30,70] have been produced by slurry dipping or electrophoretic deposition (EPD) methods. Roether and colleagues were the first to develop composites of macroporous PDLLA foams coated with Bioglass® particles (grade 45S5 with particle size <5 µm) by slurry dipping in conjunction with ethanol pretreatment [99]. A stable and homogeneous coating on the surface and infiltration of Bioglass® particles throughout the porous network were achieved. Stable slurry of 42 wt% Bioglass® in deionized water gave relatively dense and uniform adherent coatings. EPD was investigated as an alternative route whereby charged Bioglass® particles in aqueous suspension infiltrated the foam with its tubular macropores orientated perpendicularly to the larger dimension of the electrodes [99]. The slurry dipping technique was more appropriate than the EPD route, which caused sealing of the interconnected pores by Bioglass® particles. Composites tested *in vitro* in acellular SBF exhibited increasing development of HA (layers of 10 µm were formed at 28 days) and changes in pore morphology as a result of polymer degradation with increasing immersion time were observed. The *in vitro* behavior of osteoblast-like cells infiltrating these highly bioactive composites has been investigated [101]. It was demonstrated that cells were able to migrate through the porous network and colonize the lower section of the foams. Also, after 24 h a higher cell density was observed in the Bioglass® coated foams compared with the pure PDLLA foams. There is, however, no published results on the *in vivo* behavior of the composites and certainly these scaffolds should be developed and characterized further.

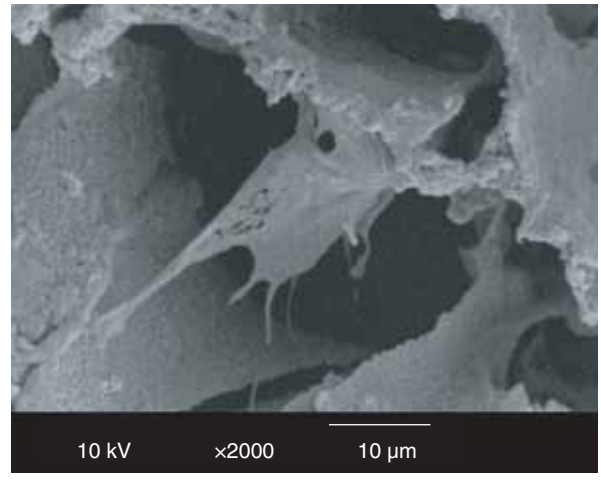

Figure 6. Scanning electron micrograph showing the well spread and flattened morphology of an MG-63 cell adhering to DL-polylactic acid/Bioglass® (40 wt%) foam after 24 h in culture [89].

Table 3. Mechanical properties of a selection of porous synthetic bioactive and biodegradable polymer and composite scaffolds.

Polymer scaffolds	Elastic modulus (MPa)	Compressive strength (MPa)	Ref.
PLLA approximately 95% porous (via TIPS)	6.2	0.24	[23]
PLLA 50wt% HA approximately 89% porous (via TIPS)	11	0.39	
PDLLA 93% porous (via TIPS)	0.9	0.08	[91]
Filled with 30wt% Bioglass®	1.2	0.08	
PLGA (75LA:25GA) 93% porous (via TIPS)	0.4	0.04	[91]
Filled with 30wt% Bioglass®	0.8	0.10	
PLGA (50LA:50GA) 31% porous (microsphere sintering)	26	0.53	[77]
Bioglass® filled 43% porous (microsphere sintering)	51	0.42	
PLLA 80% porous (via phase separation)	107		[19]
PDLLA/25wt% bioactive glass (77% porous)	145		
PDLLA/50wt% bioactive glass (78% porous)	179		

HA: Hydroxyapatite; PDLLA: DL polylactic acid; PLGA: Polylactid-co-glycolic acid; PLLA: Poly(lactic acid); TIPS: Thermally induced phase separation.

Expert opinion: needs & opportunities

The totally synthetic biodegradable polymer/inorganic bioactive phase composites reviewed here are particularly attractive as tissue engineering scaffolds due to the flexibility in tailoring structural properties, bioactive behavior and biodegradation kinetics. Significant developments have been made in extending existing polymer processing methods to the manufacturing of these synthetic polymeric matrix composites incorporating inorganic bioactive phases, as opposed to the inclusion of biomolecules or growth factors such as bone morphogenic proteins (BMPs) and VEGF, which are sensitive to many of these processing routes. Indeed, further research emphasis should be placed into the incorporation and appropriate delivery of these factors alone, or in combination with bioactive inorganic phases. For example, the possibility of incorporating bone-acting drugs or growth factors into composites formed by biodegradable polymers and bioactive glasses or HA inclusion is starting to be explored [51,102–105].

Beyond the synthetic aliphatic polyester/bioactive glass composites currently being developed, new systems appear interesting for scaffold design, in particular sol–gel derived organic/inorganic hybrids, which to our knowledge have not yet been formed into highly porous structures. Other composite systems of interest are those combining nanostructured inorganic phases (e.g., nanoparticles and nanofibres) and biodegradable polymers, recently discussed in this series [106], in order to influence cell response in contact with scaffold by mimicking the surface nanotopography of living tissues. Furthermore, the use of *in situ* porosifiers in biodegradable polymers should equip the tissue engineer with a new range of materials, including those with functionally graded microstructure and locally varying porosity, and encourage the exploration of novel fabrication procedures tailored for specific applications.

There is a lack of current understanding in the literature regarding the long-term *in vitro* characterization of the composites discussed here, specifically regarding the long-term effect of the incorporation of inorganic bioactive phases on the degradation and ion release kinetics of these highly porous systems. In this regard, the development of appropriate characterization techniques coupled with predictive analytical models is mandatory in order to be able to comprehensively assess the degradation of these systems with respect to pore structure, scaffolds geometry, fluid flow and the influence of the bioactive additions. Here, the use of x-ray microtomography as a reliable tool for quantifying 3D pore structure parameters is likely to gain increased impetus [107]. Additionally, the properties of interfaces between the polymer matrix and inorganic inclusions need to be further investigated and characterized, since interfacial properties have a large influence on degradation kinetics, ion concentration and release profiles, as well as the effective composite mechanical properties. In addition, the influence on *in vitro* media composition and inclusion of proteins in cell culture medium on the changing surface properties of these composite materials is unknown. There is significant scope in the application of surface modification and functionalization, through the use of protein adsorption or plasma treatment to provide more cues to cell attachment and response [108,109,202], especially with regard to applying these techniques to large-scale highly porous 3D systems. Adsorption of proteins such as fibronectin and collagen should cause improvements in wettability of highly hydrophobic polymers such as PDLLA. The use of improved cell seeding mechanisms and perfusion bioreactors should enable improved optimization of the scaffolds, especially with respect to the influence of ion concentration and release kinetics on cellular response. Moreover, improved cell biology assessment models need to be developed as the influence of

media composition and volume can significantly influence the ion release kinetics and cell response, which may not represent the *in vivo* model. Finally, more research needs to be directed at assessing the suitability of these bioactive composite scaffolds in soft tissue engineering strategies, including further investigation of the effect of dissolution products from the bioactive phase on vascularization. Studies have started to emerge showing that bioactive glass can be part of novel delivery devices for stimulating angiogenesis in tissue engineering strategies [30].

Five-year view

The requirements for tissue engineering scaffolds are particular to the structure and function of specific tissues concerned. Scaffold materials and fabrication techniques need to be tailored to give the desired pore structure, mechanical and bioactive properties, degradation and tissue in-growth rates, without eliciting an inflammatory response in the host tissue. This review reveals that numerous composite systems based on bioactive phases, such as HA and bioactive glasses, combined with synthetic biodegradable polymers have been developed, particularly within the last 3 years. However, processing techniques used with HA or calcium phosphate reinforced polymers have not been fully exploited for bioactive glasses and this might be a topic of further research in the near future. A most interesting development for tissue engineers appears to be in optimizing highly porous bioactive glass-filled polymer composites by tailoring their porous architectures and morphology with respect to the tissue in concern. Another promising direction is the sol-gel synthesis of polymer/silica based hybrids and exploiting foaming methods for these hybrids using the sol-gel route. Much of the research is focused on choosing adequate processing routes to provide the architecture specific to the required tissue. Thus, closer links are required between materials scientists and clinicians to develop these materials for specific applications, this being crucial for achieving success in the next 5 years. From the materials science perspective, the present challenge in tissue engineering is to design and fabricate reproducible bioactive and bioresorbable 3D scaffolds that are able to maintain their structure and integrity for predictable times, even under load bearing conditions. As emphasis should be on use of materials that activate the body's own repair mechanisms, bioactive composites as discussed in this review represent an important subset of these. Further research should focus on understanding cell–material interactions at a molecular biology level coupled with the design of a new generation of bioactive materials that activate proliferation and differentiation of cells to enhance rapid formation of extracellular matrix and tissue growth *in situ*. Inducing rapid vascular ingrowth during tissue development will continue to be of major concern for tissue engineers. Bioactive scaffolds with controlled additions of Bioglass®, a possible stimulant for angiogenic growth factor segregation, will certainly become the subject of intensive research in this context. The economic and personal benefits of *in situ* regenerative tissue repair, not only for the elderly population, will be profound.

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Key issues

- Synthetic bioactive and bioresorbable composite materials containing bioactive glass are becoming increasingly important as scaffolds for tissue engineering due to the combination of desirable properties they offer.
- Composites of tailored physical, biologic and mechanical properties, as well as, predictable degradation behavior can be produced combining synthetic bioresorbable polymers and bioactive glass. A great variety of these composites, being developed by specialized research groups worldwide is discussed.
- Strategies to improve polymer/Bioglass® scaffolds including incorporation and appropriate delivery of biomolecules and growth factors, surface functionalization, and/or design of novel micro- and nano-structures are discussed.

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- •• of considerable interest

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