CHAPTER 6

Tissue Engineering Scaffolds from Bioactive Glass and Composite Materials

Q. Chen, J. A. Roether and A. R. Boccaccini*

Summary

Bone tissue engineering combines cells and a biodegradable 3D scaffold to repair diseased or damaged bone tissue. Challenges are set by the design and fabrication of the synthetic tissue scaffold and the engineering of tissue constructs in vitro and in vivo. In bone tissue engineering, bioactive glasses and related bioactive composite materials represent promising scaffolding materials. In this chapter, we present state-of-the-art fabrication technologies for a variety of bone tissue engineering scaffolds discussing their microstructure and relevant properties. The focus is in the development of synthetic scaffolds based on bioactive glasses and their polymeric composites, including 45S5 Bioglass®, Bioglass®-poly(lactic acid) and Bioglass®-poly(hydroxylalkanoate) composites. Our group has recently developed further a number of scaffold fabrication techniques, including foam replication technique, thermally induced phase separation, textile and foam coating methods and biomimetic approaches to optimise scaffold structure and properties. Among these techniques, the foam replication method to produce highly porous, biodegradable and mechanically competent Bioglass®-derived glass-ceramic scaffolds is highlighted as one of the most promising technologies because of its potential in addressing basic scaffold requirements as well as the vascularisation issue. The enhancement of scaffold properties and functions by surface modification of the basic pore network, both its chemistry and topography, is also discussed. Finally, limitations of presently developed bone tissue constructs are summarized and future directions of research are discussed.

KEYWORDS: Scaffolds, Bioactive Glass, Composite Materials, Bone Tissue Engineering, Porosity, Nanomaterials.

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1. INTRODUCTION

An effective approach to bone tissue engineering aims to restore function to diseased or damaged bone tissue by combining isolated functional cells and biodegradable scaffolds made from engineered biomaterials. Multidisciplinary teams of scientists are working on designing and fabricating suitable scaffolds, on solving cell related issues and investigating the engineering of tissue constructs *in vitro* and *in vivo*. Currently, some of the most promising scaffolding materials for application in bone tissue engineering are bioactive glasses and related bioactive composite materials (1). These scaffolds are highly porous, three-dimensional (3D) structures exhibiting tailored porosity, pore size and interconnectivity.

Bioactive glasses are a subset of inorganic bioactive materials, which are capable of reacting with physiological fluids to form tenacious bonds to bone through the formation of bone-like hydroxyapatite layers and the biological interaction of collagen with the material surface (2). It has been found that reactions on bioactive glass surfaces lead to the release of critical concentrations of soluble Si, Ca, P and Na ions, which induce favourable intracellular and extracellular responses leading to rapid bone formation (3). Several scaffold fabrication techniques, including foam replication methods, thermally induced phase separation, textile and foam coating methods as well as biomimetic approaches to optimise the structure, properties and mechanical integrity of scaffolds have been reported in the specialised literature and recent comprehensive reviews of the state-of-the-art in the field are available (4-6). Increasing efforts are also devoted to engineer the surface of bioactive scaffolds, both in terms of chemistry and local topography which have a pronounced effect on *in-vitro* and *in-vivo* bone regeneration. In this context, the incorporation of nanotopographic features that mimic the nanostructure of natural bone is becoming an interesting area of research in tissue engineering [7, 8].

In this chapter, we present state-of-the-art fabrication technologies for a number of scaffolds being developed for bone tissue engineering, examining also scaffold microstructure (e.g. porosity and pore structure) and relevant properties. The focus is on the development of synthetic scaffolds based on bioactive glasses and their polymeric composites, including sintered 45S5 Bioglass® foams, Bioglass®-poly(D, L-lactic acid) (PDLLA) and Bioglass®-poly(hydroxylalkanoate) (PHA) composites. The enhancement of scaffold properties and functions by surface modification of the basic pore network, both the chemistry and topography, for example using carbon nanotubes (CNTs), is also discussed. Finally, the limitations of

presently developed bone tissue constructs are summarized, and areas for future research are highlighted.

2. SCAFFOLDS REQUIREMENTS

Bone tissue engineering seeks to restore and maintain the function of human bone tissues using the combination of cell biology, materials science and engineering principles. The three main ingredients for tissue engineering are therefore, harvested cells, recombinant signalling molecules, and 3D matrices. Cells and signalling molecules such as growth factors are seeded into highly porous biodegradable scaffolds, cultured *in vitro*, and subsequently the scaffolds are implanted into bone defects to induce and direct the growth of new bone. Signalling molecules can be coated onto the scaffolds or directly incorporated into them. Hence, the first and foremost function of a scaffold is its role as the substratum that allows cells to attach, proliferate, differentiate (i.e., transform from a non-specific or primitive state into cells exhibiting the bone-specific functions), and organize into normal, healthy bone as the scaffold degrades. Figure 1 (4) summarises most important factors involved in the optimised design of tissue engineering scaffolds.

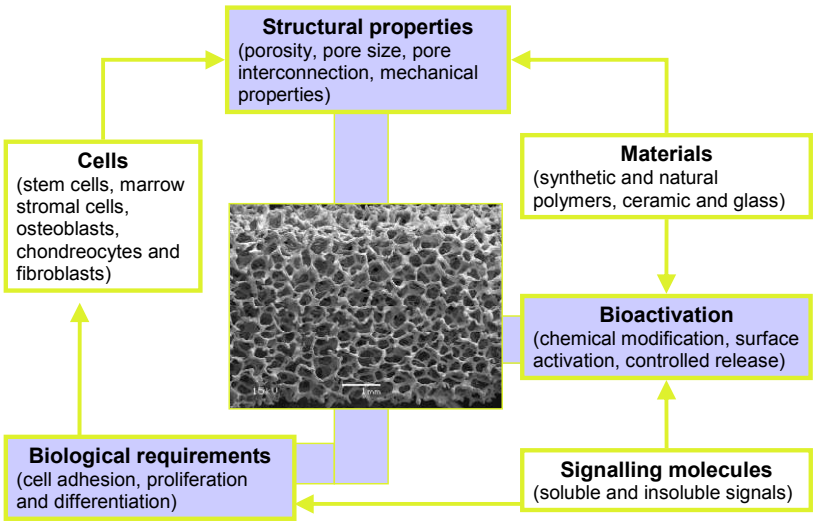

Fig. 1. Most important factors involved in the design of optimal scaffolds for bone tissue engineering (Modified after ref. (4]).

Scaffolds for bone tissue engineering are subject to many interlinked and often opposing biological and structural requirements, which are summarised in Table 1. A major hurdle in the design of tissue engineering scaffolds is that most materials are not simultaneously mechanically competent and bioresorbable, i.e. mechanically strong materials are usually bioinert, while degradable materials tend to be mechanically weak (9). Hence, the fabrication of composites comprising biodegradable polymers and bioactive glass becomes a suitable option to fulfil the requirements of bioactivity, degradability and mechanical competence.

Table 1. Design criteria for bone tissue engineering scaffolds (1, 4, 10, 11).

1. **Ability to deliver cells**
   The material should not only be biocompatible (i.e. harmless), but also foster cell attachment, differentiation, and proliferation.
2. **Osteoconductivity**
   It would be best if the material encourages osteoconduction with host bone. Osteoconductivity does not only eliminate the formation of fibrous tissue encapsulation but it also brings about a strong bond between the scaffold and host bone.
3. **Biodegradability**
   The composition of the material, combined with the porous structure of the scaffold, should lead biodegradation *in vivo* at rates appropriate to tissue regeneration.
4. **Mechanical properties**
   The mechanical strength of the scaffold, which is determined by both the properties of the biomaterial and the porous structure, should be sufficient to provide mechanical stability to constructs in load bearing sites prior to synthesis of new extracellular matrix by cells.
5. **Porous structure**
   The scaffold should have an interconnected porous structure with porosity > 90% and diameters between 300-500 μm for cell penetration, tissue ingrowth and vascularisation, and nutrient delivery.
6. **Fabrication**
   The material should possess desired fabrication capability, e.g., being readily produced into irregular shapes of scaffolds that match the defects in bone of individual patients.
7. **Commercialisation potential**
   The synthesis of the material and fabrication of the scaffold should be suitable for commercialisation.

3. SELECTION OF BIOMATERIALS FOR BONE TISSUE ENGINEERING SCAFFOLDS

Since natural bone matrix is a composite of biological ceramic (natural apatite) and biological polymer (collagen), it is not surprising that synthetic or naturally occurring ceramics, polymers,

and their composites have been extensively considered to construct scaffolds for bone tissue engineering [1, 4-6]. Some basic characteristics of these materials are discussed in the following paragraphs.

3.1. Bioceramics and bioactive glasses

Since bone consists of large amounts of hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$, HA and related calcium phosphates (CaP) (e.g., β-tricalcium phosphate) have been considered to develop scaffold materials for bone regeneration. Due to their close chemical and crystal resemblance to the mineral phase of bone HA and CaP exhibit excellent biocompatibility. The close similarity of hydroxyapatite to the mineral component of bone, which is stable in the body, results however in the lack of biodegradation of HA in the body, which is generally an undesirable feature for tissue engineering scaffold materials. For example, a recent clinical report on a 6-7 year follow-up study has confirmed that implanted crystalline HA is not biodegradable, remaining in the body for extended periods with no visible signs of biomaterial resorption (12).

Bioactive silicate glasses (e.g. 45S5 Bioglass®) with compositions in the system SiO_2-Na_2O-CaO-P_2O_5, having <55% SiO_2 were discovered by Hench in 1969 [2]. They offer remarkable advantages as the inorganic components of composite scaffolds due to their high bioactivity index (Class A), and their ability to bond to both soft and hard connective tissues (13). Class A bioactive materials are osteogenetic and osteoconductive materials while Class B bioactive materials (such as hydroxyapatite) exhibit only osteoconductivity. It has also been found that reactions on bioactive glass surfaces release critical concentrations of soluble Si, Ca, P and Na ions, which induce intracellular and extracellular responses (3). For example, a synchronised sequence of genes is activated in osteoblasts that undergo cell division and synthesise an extracellular matrix (ECM), which mineralises to become bone (3, 14). In addition, 45S5 Bioglass® has been shown to increase the secretion of vascular endothelial growth factor (VEGF) *in vitro* and to enhance vascularisation *in vivo*, suggesting scaffolds containing controlled concentrations of Bioglass® might stimulate neo-vascularisation which is beneficial to large tissue engineered constructs (15).

The excellent properties of bioactive glasses and their long history of applications in biomedical implants (2) have prompted extensive research in the last 10 years regarding their use in bone engineering and regeneration strategies. Although bioactive glasses are mechanically

weak, it has recently been discovered that 45S5 Bioglass® can partially crystallise when heated to high temperatures (> 950 ºC) during scaffold fabrication and that the mechanically strong crystalline phase can transform to a biodegradable, amorphous calcium phosphate at body temperature and in a biological environment (16,17). This transformation enables the two normally irreconcilable properties, i.e. mechanical competence and biodegradability, to be combined in a single scaffold. This discovery promises to go some way towards the scaffold optimisation and its clinical application.

3.2. Naturally occurring polymers

Theoretically, naturally occurring polymers should not cause foreign material response when implanted in humans. They also provide a natural substrate for cellular attachment, proliferation and differentiation and are considered favourite substrates for tissue engineering (18). However, their poor mechanical properties and variable physical properties with different sources of the protein matrices have hampered progress with these materials. Concerns have also arisen regarding immunogenic problems associated for example with the introduction of foreign collagen (19).

The drawbacks associated with naturally occurring polymers could be averted with polyhydroxyalkanoates (PHAs), aliphatic polyesters produced by microorganisms under unbalanced growth conditions (20). They are generally biodegradable (via hydrolysis), highly biocompatible, and thermo-processable, being thus attractive for applications in tissue engineering (21). The blending among the several PHAs can dramatically change material properties and biocompatibility. Over the past years, PHAs, particularly poly 3-hydroxybutyrate (PHB), copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV), poly 4-hydroxybutyrate (P4HB), copolymers of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx) and poly 3-hydroxyoctanoate (PHO) have demonstrated suitability for tissue engineering and are reviewed in detail in references (21, 22).

PHB is of particular interest for bone tissue engineering considering that a consistent favourable bone tissue adaptation response was demonstrated with no evidence of undesirable chronic inflammatory response after implantation periods up to 12 months (23). A possible drawback of some PHAs, however, is their limited availability and the time consuming extraction procedure from bacterial cultures that is required for obtaining sufficient amounts, as

described in the literature (21). Therefore, the extraction process might be a challenge to a cost effective industrial upscale production for large amounts of some PHAs.

3.3. Synthetic polymers

A great deal of research effort has gone into developing synthetic polymers as tissue engineering scaffolds. Synthetic polymers have numerous advantages, such as excellent processing characteristics, which can ensure the off-the-shelf availability as well as being biocompatible and biodegradable at rates that can be tailored for the intended application (19, 24). Additionally, synthetic polymers possess predictable and reproducible mechanical and physical properties (e.g. tensile strength, elastic modulus, and degradation rate) and can be manufactured with great precision. On the other hand, many such polymers suffer shortcomings, such as eliciting persistent inflammatory reactions, being eroded, not being compliant or capable to integrate with host tissues.

Between the two types of synthetic polymers, i.e. bulk biodegradable and surface bioerodible polymers, the former have shown more promise considering that one of the requirements of a tissue engineering scaffold is that it has to be replaced by newly formed bone tissue *in vivo*. Among the bulk degradable polymers, amorphous poly(D,L- lactic acid) (PDLLA) is one of the most popular materials considered for scaffold production, also in combination with bioactive glasses (25), because it can be combined with biomolecules, such as growth factors (26) and antibiotics (27), to establish a locally acting drug-delivery system. It is expected that a scaffold with a controlled drug-delivery function will promote bone regeneration and eliminate possible inflammatory responses upon scaffold degradation.

3.4. Composites

From a biological perspective, it makes sense to combine polymers and bioceramics to fabricate scaffolds for bone tissue engineering because native bone is the combination of a naturally occurring polymer and biological apatite. From the materials science point of view, a single material type does not usually provide the necessary mechanical and/or chemical properties required (Table 1) hence the properties of two or more materials can be combined in a composite material. Polymers and ceramics (and glasses) that have the ability to degrade *in vivo* are ideal candidates for composite scaffolds which gradually degrade while new tissue is formed. While

massive release of acidic degradation from polymers can cause inflammatory reactions (28, 29), the basic degradation of calcium phosphate or bioactive glasses could buffer the acidic by-products of polymers thus contributing to avoid the formation of an unfavourable environment for cells due to low pH values.

Mechanically, bioceramics and glasses are stronger than polymers and play a critical role in providing mechanical stability to constructs prior to synthesis of new bone matrix by cells. However, ceramics and glasses are very fragile and prone to catastrophic failure due to their intrinsic brittleness and flaw sensitivity. The formation of composites thus capitalises on the advantages of both material types and minimise their shortcomings. One major challenge to optimise the biological and mechanical performance of bioactive polymer/ceramic composites is to obtain good chemical and/or physical bonding between the polymer and the inorganic phase. It is worthwhile mentioning that composites are also the materials of choice for use in tissue engineering strategies to repair osteochondral defects, i.e. when subchondral bone as well as cartilage, synovium and joint capsule, are damaged as a result of degenerative diseases such as osteoarthritis (30). In this case, a simultaneous regeneration of both cartilage and subchondral bone is desired using bi-phasic (or layered) composite scaffolds to guide the simultaneous regeneration of both tissues.

Table 2 lists selected typical biodegradable and bioactive ceramic/glass-polymer composites, which have been designed for bone tissue engineering scaffolds, and their mechanical properties. One important group of composite scaffolds reported in literature comprises tailored combinations of Bioglass® particles and biodegradable polymers (e.g. PLGA, PDLLA, PHB) (6, 25, 31-51), which have shown high application potential. These composites have a well-defined porous structure, at the same time their mechanical properties are close to those of cancellous bone (52-54) and the high bioactivity is conferred by the Bioglass® particulate filler.

Stronger composite scaffolds might be achievable by increasing the organic/inorganic interfacial bonding by using for example surface functionalized particles. A higher degree of particle loading is generally directly proportional to increases in stiffness, however the increase in particle loading also increases the number of interfaces which may give rise to more fracture surfaces along which cracks can propagate. A number of studies suggest that well-dispersed nanostructured composites may offer surface and/or chemical properties closer to native bone,

and therefore they might represent ideal substrates to support bone regeneration (7, 55, 56). Nanosized bioactive glass particles have become recently available which can be considered as ideal fillers for tissue engineering scaffolds (57). However, problems associated with poor interfacial bonding and particle agglomeration may be more pronounced when using nanosized particles. To improve the bonding between inorganic particles and matrix silane coupling agents have been employed as well as titanates and zirconates (58, 59).

Table 2. Biodegradable and bioactive composites for bone tissue engineering.

Biocomposite		Percentage of ceramic (%)	Porosity (%)	Pore size (μm)	Compressive(C), Tensile (T), Flexural (F) Strength (MPa)	Modulus (MPa)	Ref.
Ceramic	Polymer						
Non-crystalline CaP	PLGA	28-75 (wt.)	75	> 100		65	(31, 32)
β-TCP	Chitosan-Gelatin	10-70 (wt.)		322-355	0.32-0.88 (C)	3.94-10.88	(33)
β-TCP	PLGA	30 (wt.)		400 (macro) 10(micro)	-	-	(34)
HA	PLLA	50 (wt.)	85-96	100×300	0.39 (C)	10-14	(35)
	PLGA	60-75 (wt.)	81-91	800-1800	0.07-0.22 (C)	2-7.5	(36)
	PLGA		30-40	110-150	-	337-1459	(37)
nHA	PA	60 (wt)	52-70	50-500 (macro) 10-50 (micro)	13.20-33.90(C)	0.29-0.85	(38)
HA	PCL	25 (wt)	60-70	450-740		76-84	(39)
HA	PLAGA	50-87(wt.)			80 (C)	Up to 120	(40)
Bio-glass®	PLGA	75 (wt.)	43	89	0.42 (C)	51	(41-43)
	PLLA	20-50 (wt.)	77-80	~100 (macro) ~10 (micro)	1.5-3.9 (T)	137-260	(44)
	PLGA	0.1-1 (wt.)		50-300			(45)
	PDLLA	5-29 (wt.)	94	~100 (macro) 1050 (micro)	0.07-0.08	0.65-1.2	(46-48)
CaP glass	PDLLA	20-50 (wt.)	93%-96.5%	80-450	-	0.05-0.2	(49)
A/W Phosphate Glass	PLA-PDLLA	40 (wt.) 20-40 (wt.)	93-97 85.5-95.2	98-154	0.017-0.020 (C)	0.075-0.12	(50, 51)
	PDLLA						
Human Cancellous Bone					4 -12 (C)	100-500	(52-54)

4. FABRICATION TECHNOLOGIES OF BONE TISSUE ENGINEERING CONSTRUCTS FROM BIOACTIVE GLASSES AND COMPOSITE MATERIALS

4.1. 3D bioactive glass scaffolds

4.1.1. Sol-gel process

Sol-gel process is defined as the chemical synthesis of inorganic materials by preparation of a sol, gelation of the sol (gel) and removal of the solvent. The sol-gel process involves the transition of a system from a liquid "sol" into a solid "gel" phase. The chemistry involved in the process is based on inorganic polymerisation reactions of metal alkoxides.

Highly porous glasses (or glass foams) have been developed by directly foaming the sol using a double blade mixer, a surfactant and an acidic catalyst (dilute HF) added as gelling agent (60-62). The precursors of the glass foams are $Ca(NO_3)_2$ and two alkoxides: tetraethylorthosilicate (TEOS) and triethylphosphate (TEP). A hierarchical structure can be obtained, with mesopores (2-50 nm) for enhanced reactivity and cell attachment and an interconnected array of macropores (10-500 μm) for tissue ingrowth. These macro-porous glasses provide the potential properties for applications in tissue engineering and *in situ* bone tissue repair and regeneration. They have shown favourable results in both *in vitro* and *in vivo* tests for bone regeneration (63).

4.1.2. Foam replica technique

The foam replica technique is a process originally developed for the manufacture of ceramic foams in 1963 (64). The adaptation of the process to fabricate Bioglass® scaffolds is shown schematically in Figure 2.

In the polymer-replication process, the starting structure (green body) is prepared by coating a polymer (e.g., polyurethane) foam with bioactive glass (Bioglass®) particles by slurry infiltration. The polymer foam, already having the desired macrostructure, serves as a sacrificial template for the bioactive glass coating. The polymer template is immersed in the slurry, which subsequently infiltrates the structure leading to a homogeneous coating of Bioglass® particles on the surface of the polymer substrate. After drying, the polymer is slowly burned out at high temperature (> 450 ºC) in order to minimise microstructure damage (i.e. microcracking) of the

porous Bioglass® coating. Once the polymer has been removed, the glass is sintered to the desired density. The process replicates the macroporous structure of the polymer foam, and results in a rather distinctive microstructure of the struts, as shown in Figure 3.

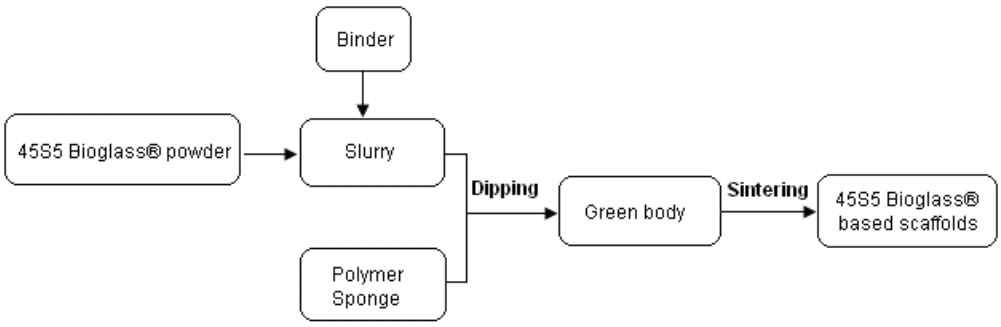

Fig. 2. Schematic diagram showing the foam replica method to fabricate Bioglass® tissue engineering scaffolds (16).

The foam replica technique has a number of advantages over other scaffold fabrication techniques, such as the ability to produce foams with a highly porous structure with adjustable pore dimensions. Moreover irregular shapes can be produced to match the size and shape of the bone defect. Additionally, the foam replication technique does not involve the use of toxic chemicals and is more rapid and cost effective compared to other standard processing techniques such as SFF rapid prototyping.

Figure 3 shows the connective, open porous structure of a scaffold made from 45S5 Bioglass® using the replication technique (16). The porosities of the scaffolds are in general higher than 90%, with the pore size being 500-700 μm. The scaffolds, which are sintered at a temperature above 1000°C, have shown compressive and bending strengths that are higher than those of equivalent hydroxyapatite foams with similar porosities reported in literature (65, 66). This improved mechanical strength was attributed to the fine crystalline particles ($Na_2Ca_2Si_3O_9$ crystals) formed during sintering which lead to a typical glass-ceramic microstructure of the foams (16, 17). More significantly, the mechanically strong crystalline structure is able to transform to amorphous and thus biodegradable calcium phosphate in a biological environment (17).

In vitro investigations have shown that the Bioglass®-derived glass-ceramic scaffolds have excellent osteoblast cell-support ability. Cells infiltrate effectively into the porous structure

and proliferate in the central region of the highly porous scaffolds (67). The ability of the Bioglass®-derived scaffolds to deliver cells could be enhanced further by surface functionalisation (silanisation), as demonstrated recently (67, 68).

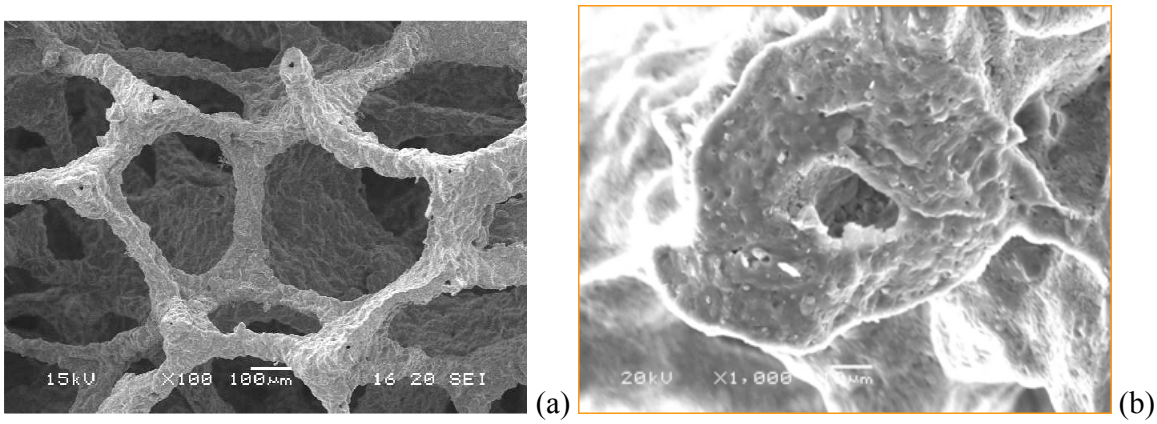

Fig. 3. Scanning electron microscopy (SEM) images of a Bioglass® glass-ceramic scaffold fabricated by the foam replica method: (a) low magnification image showing interconnected pore structure and large porosity, (b) high magnification image showing the cross section of a foam strut (Micrographs courtesy M. Darmawati Yunos and O. Bretcanu, Imperial College London).

4.2 Polymer coated Bioglass® scaffolds

In order to improve the mechanical stability of highly porous ceramic scaffolds, many authors have investigated the coating of the scaffolds with biodegradable polymers (69-72). For the particular case of Bioglass® based scaffolds, both PDLLA (69) and PHB (70) have been considered. Chen *et al*. (69), for example, coated Bioglass®-derived foams with PDLLA by a slurry immersion procedure, schematically shown in Figure 4. It was found that the work-of-fracture of the foams after PDLLA coating was significantly enhanced, being 20 times higher than the value without PDLLA coating. The polymer layer was made to cover and fill the microcracks situated on the strut surfaces, improving the mechanical stability of the scaffold as the polymer layers induced a crack bridging mechanism, which is considered to be similar to the effect of collagen fibrils on the fracture process of natural bone (73, 74). It has also been found that upon immersion of PDLLA coated Bioglass® foams in simulated body fluid, HA crystals formed inside the polymer coating layer (69). Eventually, the surface of the foams develops a nanostructured composite layer leading to improved mechanical integrity of the construct. The mechanical strength of as-sintered foams decreased to a large extent (from 0.3 to 0.03 MPa) upon immersion of the foams in simulated body fluid when the crystalline phase $Na_2Ca_2Si_3O_9$

transformed to amorphous calcium phosphate. However, the mechanical performance can be maintained in polymer coated foams even after immersion in simulated body fluid for eight weeks when the crystalline phase $Na_2Ca_2Si_3O_9$ transformed to the amorphous calcium phosphate (69, 70).

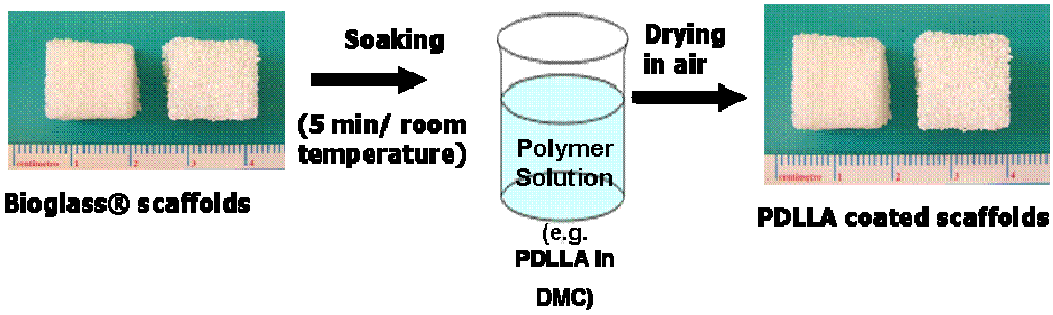

Fig. 4. The polymer solution dipping method developed to coat Bioglass® scaffolds with PDLLA (69) or PHB (70).

4.3. Polymer composite scaffolds

While intensive efforts have been made to develop processing technologies for polymer and ceramic scaffolds, less attention has been paid to the fabrication of porous composite scaffolds. Among a number of polymer processing techniques (1, 4, 5, 41), solvent casting with and without particle leaching (36, 41, 44, 50, 51), thermally induced phase separation (TIPS) combined with freeze-drying (6, 33, 35) and solid free form fabrication (5) have been applied successfully to the fabrication of polymer-ceramic composite scaffolds as discussed next.

4.3.1. Solvent Casting

Solvent casting of the composite scaffolds involves the dissolution of the polymer in an organic solvent, mixing with bioactive ceramic or glass granules, and casting the solution into a predefined 3D mould. The solvent is subsequently allowed to evaporate. The main advantage of this processing technique is the ease of fabrication without the need of specialised equipment. The primary disadvantages of solvent casting are (1) the limitation in the shapes (typically flat sheets and tubes are the only shapes that can formed); (2) the possible retention of toxic solvent within the polymer; and (3) the denaturation of the proteins and other molecules incorporated into the polymer by the use of solvents. The use of organic solvents to cast the polymer may

decrease the activity of bioinductive molecules (e.g., protein). Detailed processing steps can be found in Ref. 41 (41).

4.3.2. Solvent Casting / Particle Leaching and Microsphere Packing

Bioactive polymer-ceramic constructs can be fabricated by the combination of solvent casting, particle leaching and microsphere packing methods. Polymer microspheres are firstly formed from traditional water oil/water emulsions. Polymer-bioceramic scaffolds can then be constructed by mixing solvent, salt or sugar particles (porogens), bioactive glass or ceramic granules and pre-hardened microspheres (75). A 3D structure of controlled porosity is formed based on this method combined with particle leaching and microsphere packing. This method shares similar advantages and disadvantages with the solvent casting technique. For details of the method, readers may refer to Ref. 41 (41).

4.3.3. Thermally Induced Phase Separation / Freeze-Drying

Porous composite structures can be attained through thermally induced phase-separation (TIPS) and evaporation. One approach to induce phase separation is to lower the temperature of the suspension of polymer and inorganic materials. The solvent is solidified first, forcing the polymer and ceramic mixture into the interstitial spaces. The frozen mixture is then lyophilised using a freeze-dryer, in which the ice solvent evaporates (6, 76).

The TIPS method can produce homogeneous and highly porous (~95%) scaffolds with highly anisotropic tubular morphology and extensive pore interconnectivity (6, 25, 46, 48, 76). The pore morphology varies depending on the polymer, solvent, concentration of the polymer solution and phase separation temperature. Foams obtained from this process usually exhibit oriented tubular pores of diameters of several hundred microns (>100μm) and isotropic pore network of smaller pore size (~ 10 μm) connecting the large tubular pores (6). The TIPS process has been used to produce composite scaffolds based on PLGA and PDLLA foams containing Bioglass® particles (6, 25, 46, 48) and a cross section of a PDLLA/Bioglass® scaffold developed by this method is shown in Figure 5 (77). The possibility of coating TIPS produced foams with Bioglass® particles has also been investigated (78).

Due to the potential advantages the PDLLA/Bioglass® composite system offers, there has been recent increased interest in investigating its in vivo and in vitro response (79-81).

PDLLA/Bioglass® films were demonstrated to enhance bone nodule formation and displayed enhanced alkaline phosphatase activity of primary human fetal osteoblasts in the absence of osteogenic supplements (79). The attachment and spreading of osteoblast cells onto PDLLA/Bioglass® 3D composite foams has been also confirmed (46). Moreover, Helen *et al*. (81) have shown that composite PDLLA/Bioglass® films are an appropriate substrate for the culture of annulus fibrous cells *in vitro* and have proposed the composite as a suitable material for intervertebral disc tissue repair.

4.3.4. Microsphere-Sintering

In this process, microspheres formed by a polymer matrix and bioactive glass or ceramic inclusions are first synthesized using a variety of techniques including the spraying of polymer solutions followed by non-solvent induced phase separation (NIPS). Lu *et al.* (43) have worked on this technique using PLGA and Bioglass® as the starting materials. Once the composite microspheres have been synthesized, sintering, generally without the application of pressure, is employed in 3D moulds to yield 3D, porous composite scaffolds (31, 32, 40). More details on the process can be found in a recent review (82).

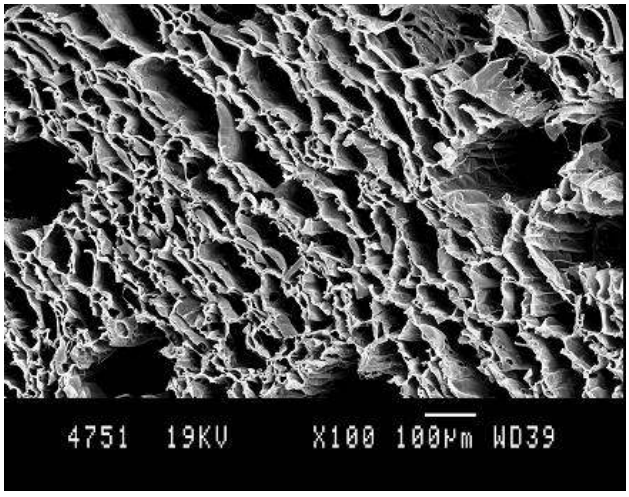

Fig. 5. Scanning electron microscopy image of the cross section of PDLLA/Bioglass® composite scaffold produced by the TIPS method (77).

4.3.5. Polymeric Foam – Inorganic Coating

An alternative approach to address the combination of biodegradable polymers and bioactive glass or ceramic materials is to coat the inorganic particles onto polymeric foams (78). For

example, porous polymeric scaffolds have been coated with bioactive glasses and other inorganic particles by slurry dipping or electrophoretic deposition methods (77, 78, 83). Roether *et al*. (78) were the first to develop composites of macroporous polymeric scaffolds (fabricated by TIPS) coated with bioactive glass particles by slurry dipping in conjunction with ethanol pre-treatment. A stable and homogeneous coating on the surface and infiltration of Bioglass® particles throughout the porous network were achieved, as shown in Figure 6 (78).

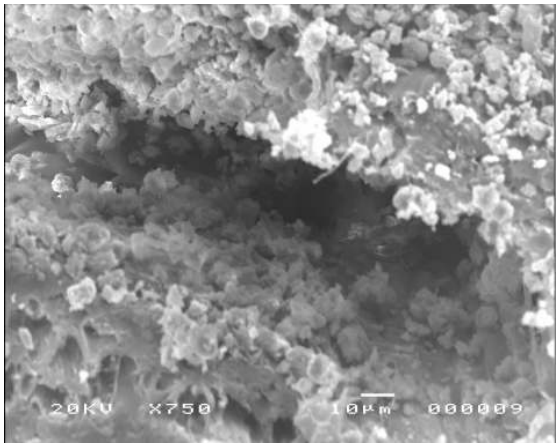

Fig. 6. Scanning electron microscopy image of the pore surface in a PDLLA foam coated with Bioglass® particles (78).

Electrophoretic deposition (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, as schematically shown in Figure 7 (78). Composites tested *in vitro* in acellular SBF exhibited increasing development of HA and changes in pore morphology as a result of polymer degradation with increasing immersion time. The *in vitro* behaviour of osteoblast-like cells infiltrating these highly bioactive composite scaffolds has been investigated (84). It was demonstrated that cells were able to migrate through the porous network and colonised the lower section of the foam. The coating of biodegradable polymer substrates with inorganic bioactive particles has been also investigated as part of so-called biomimetic strategies (85, 86). 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.

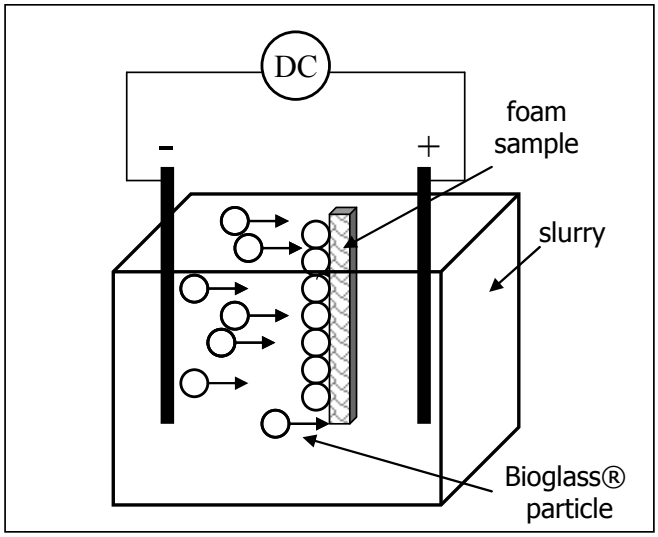

Fig. 7. Schematic diagram of the electrophoretic deposition (EPD) cell used to coat and infiltrate PDLLA foams with charged Bioglass® particles in aqueous suspension (78)

4.3.6. Solid freeform (SFF) techniques

A number of solid freeform fabrication (SFF) techniques including 3D printing, selective laser sintering, multi-phase jet solidification, and fused deposition modeling (FDM) have been developed to manufacture tissue scaffolds for bone tissue engineering with specific designed properties (5, 34, 39, 87). The scaffolds have a high degree of interconnectivity and the porosity can be controlled to a great extent by optimising the processing parameters. SFF techniques offer a unique opportunity to study the influence of the micro-architecture of the scaffold upon cell proliferation and ECM generation. The methods can furthermore be used to create scaffolds that both incorporate patient-specific information as well as an explicitly designed micro-environment. Tissue geometry can be extracted from patient's computed tomography (CT) or magnetic resonance imaging (MRI) data and reconstructed as a 3D model. Additionally, as with most computer-aided design, analysis of the mechanical and transport properties can aid in the understanding of tissue growth in a scaffold-guided environment. Among different SFF methods, FDM has recently attracted more interest due to its ability to form 3D structures by layer-by-layer deposition. The system utilizes a filament of thermoplastic material that is fed into a liquefying chamber by two rollers. These rollers provide the necessary pressure to extrude the molten composite material out through a nozzle tip (34, 39). However, the time consuming precursor step of filament fabrication can act as a main obstacle for these processes and further developments in the field are expected [39].

5. NANOSCALE ENGINEERING OF SCAFFOLDS

There has been growing interest in using nanomaterials in bone tissue engineering scaffolds in recent years in order to mimic the structure of natural bone tissue which possesses a nanocomposite structure interwoven in a 3D matrix (7, 55, 56, 88, 89). The inclusion of nanoparticles into the biopolymer matrix has the dual objective of improving the mechanical properties as well as of incorporating nanotopographic features that mimic the nanostructure of natural tissue (7, 88-91). As discussed by Berry *et al*. (92), the role of the scaffolds is being extended from being a mere mechanical support to include intelligent surfaces capable of providing both chemical and physical signals to guide cell attachment and spreading, possibly influencing also cell differentiation (see also Figure 1).

Recently, research has focussed on incorporating carbon nanotubes (CNTs) into the scaffold structure, due to the functionalities CNTs can provide, which include improved tracking of cells, electrical conduction and sensing of microenvironments, delivering of transfection agents, and mechanical reinforcing of the scaffold (90, 93, 94). Moreover using carbon nanotubes for optical, magnetic resonance and radiotracer contrast agents would provide better means of evaluating tissue formation. In addition, monitoring and altering intra and intercellular processes would be useful for the design of better engineered tissues (95). The incorporation of CNTs is also useful to tailor the (nano) roughness and topography of scaffold pore surfaces which have a profound effect on early cell attachment behaviour as well as possibly on subsequent cell adhesion, cytoskeletal organization and gene expression (92). It is now accepted that the response of host tissue at the protein and cellular level to nanostructured surfaces is different than that observed to conventional (μm) surfaces (7, 88). It is also recognised that CNTs have the potential for providing enhanced structural reinforcement in a polymer matrix at very low concentrations (to counterbalance the fact they are non-degradable).

There has been only limited work on combining bioactive glasses and CNTs in novel composite scaffolds (90, 96). Chicatun *et al*. (96) showed that nanostructured CaP deposits could be observed on the surface of CNT coated Bioglass® scaffolds exposed to simulated body fluid which were not observed on uncoated scaffolds. The SEM micrograph in Figure 8 shows a Bioglass® surface coated with a CNT mesh obtained by electrophoretic deposition fabricated by the authors.

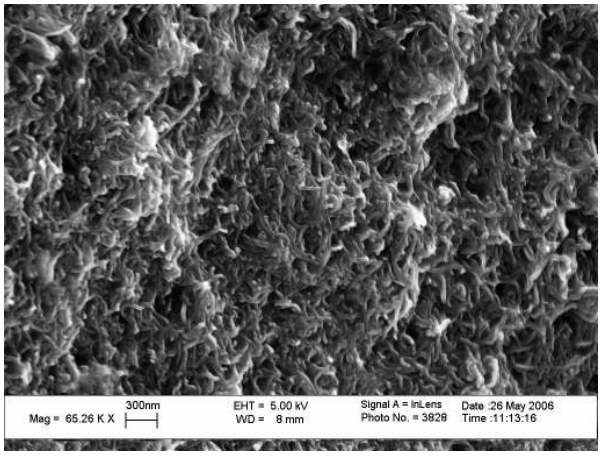

Fig. 8. Coating of (multi-walled) CNTs on the surface of a sintered Bioglass® substrate obtained by electrophoretic deposition (96).

The addition of CNT is expected to add extra functionalities to the scaffold, for example a sensing function exploiting the electrical conductivity of CNT would be possible or the release of bioactive factors by CNT functionalization (93). However, issues related to the cytotoxicity of CNT (and of nanoparticles in general) remain unresolved and they should be investigated in parallel as there are controversial reports and debates in the literature. Potential cytotoxic effects associated with carbon nanotubes may be mitigated by chemically functionalizing the surface. A review on the topic has recently been published (93).

6. SUMMARY AND FURTHER WORK

Being a relatively fledgling discipline, tissue engineering encounters a variety of challenges, which are associated with the science and technology of cells, materials, and interaction between them. The challenges that the material scientists encounter are linked with the complex combination of properties required for optimal scaffolds. An ideal scaffold should mimic the ECM of the tissue to be restored. When designing a biocomposite scaffold a large hurdle is the engineering of the interfacial characteristics, and more research efforts need to be focussed on this aspect.

For bone regeneration, the biggest challenge is the fabrication of scaffolds exhibiting suitable mechanical properties to replace large (critical size) cortical bone defects and capable of load transmission. Although a number of materials and fabrication techniques have been developed, several issues need to be addressed prior to clinical application, such as mechanical

reliability of scaffolds, induction of vascularisation and tailored degradability. The incorporation of biomolecules such as growth factors with the aim to accelerate local bone healing is promising and it is currently under extensive research. Moreover, there is significant scope in the application of surface modification, through the use of protein adsorption or plasma treatment, to provide more cues to cell attachment and response, thus making the scaffold more biocompatible.

There is limited understanding regarding the long-term *in vitro* and *in vivo* characterization of porous 3D composite scaffolds, 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 of prime importance in order to be able to comprehensively assess the degradation of these systems with respect to pore structure, scaffold's geometry, fluid flow and the influence of bioactive additions. In this respect, the use of X-ray microtomography as a reliable tool for 3D pore structure quantification is likely to gain increased impetus. Finally, in order to target clinical applications, *in vivo* studies are inevitable and the need for more research on composite scaffolds in realistic biological systems is imperative. This includes also research directed at assessing the suitability of bioactive composite scaffolds for enhancing *in vivo* angiogenesis and vascularization of tissue/scaffold constructs.

A combinatory approach, possibly using stem cells, signalling molecules and novel functional biomaterials to enhance cell growth and proliferation, to encourage vascularisation and to support damaged bone, will be needed to bring bone tissue engineering into clinical application. In this approach, engineered composite scaffolds made by smart combination of biodegradable polymers and bioactive glasses, as reviewed in this chapter, will play a vital role and they might represent the "scaffolds of choice" in combination with stem cell seeding. The use of nanomaterials such as ceramic nanoparticles and carbon nanotubes (CNTs) may also improve the environment to enhance cell attachment and proliferation as well as adding extra functionalities to the base scaffold, however possible toxicity issues associated with nanoparticles and CNTs will have to be comprehensively addressed.

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