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New trends in bioactive scaffolds: The importance of nanostructure

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Abstract

There are many criteria for an ideal scaffold that will stimulate the body's repair mechanisms to regenerate diseased or damaged bone to its original healthy state. These include having a pore network large and open enough for cells and blood vessels to penetrate and the ability to bond to bone. Sol–gel derived bioactive glasses have a nanoporosity that can control degradation rate. They can be foamed to produce scaffolds that mimic cancellous bone macrostructure. Bioactive glass foams with optimised nanoporosity are strong in compression; however, they have low toughness and pore strength when loaded in tension. Therefore an ideal scaffold would have all the properties of the glasses with enhanced toughness. This can only be achieved by creating new nanoscale composites. Resorbable polymers must interact with the silica based inorganic network at the nanoscale to maintain bioactivity and controlled resorption. This is a complex problem but may be the future of scaffold development.

Keywords: Sol–gel processes; Nanocomposites; Porosity; Biomedical applications; Scaffolds

1. Introduction

Due to increasing life expectancy, we are now outliving our body parts, including our bones. Current surgical techniques involve the use of transplants, which are in short supply, and implants that replace or augment (support) tissue, rather than regenerating it. Bone can heal itself if the defect is small, but it cannot if it is large. The bone cells need a stimulatory framework. There is therefore a need for materials that are available to surgeons off the shelf that can act as templates (scaffolds) and can stimulate the body's regenerative mechanisms. This strategy is termed bone regeneration. There are many design criteria for an ideal scaffold for bone regeneration and no one material or scaffold has yet been developed that fulfils all of them. Simplistically, scaffolds are often designed to mimic the structure and properties of the organ which they are to replace.[1,2]

However mimicking bone is complex as bone has a complicated hierarchical structure. It has two macrostructures: dense cortical bone and a supporting structure of cancellous bone which is an open macroporous network.[3] Its nanostructure consists of a matrix of collagen fibrils that provides tensile strength and toughness, some other proteins (∼5%), and hydroxycarbonate apatite (HCA) nanocrystals.[1] A successful scaffold will mimic this structure and will resorb completely over time, promoting full bone regeneration with no evidence of scar tissue. Therefore the material must not only stimulate and support tissue growth in three dimensions, but it must also degrade at the rate at which new tissue forms, and importantly, it must also have the additional ability to withstand the loading conditions experienced *in situ*. The mechanical support must continue as the material degrades, until the new tissue can take up the load.

A drawback to this strategy is that the level of detail in the molecular structure of bone is unmatched outside the biological world.[1] It is clear, therefore, that engineers will have to make some compromises in the material design and allow the body to remodel the bone once it has filled the defect and the scaffold degrades. In fact this is a similar mechanism to how bone is thought to repair naturally, with the body first producing immature (woven) bone, which is then remodelled into mature structural bone.[3] Therefore in scaffold design the engineer must consider the characteristics of the scaffold from macro scale down to the nano and atomic scale in order to be successful.

2. The criteria for an ideal scaffold

For a bone regenerating scaffold to be successful for direct implantation in any type of bone defect, it must[4]:

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1. be biocompatible (i.e. induce minimal toxic or immune response *in vivo*);
2. promote cell adhesion, bond to bone and stimulate osteogenesis;
3. act as a template for bone growth and therefore have an interconnected porous structure that can allow cellular ingrowth, vascularisation and a supply of nutrients;
4. resorb safely in the body and have a controllable degradation rate;
5. exhibit mechanical properties similar to that of the host bone;
6. have a fabrication process which allows the scaffold to be shaped to fit a range of defect geometries; and
7. be sterilisable and meet the regulatory requirements for clinical use.

The ideal specific morphology for the pore network is unclear. What is clear is that the interconnect size is the most important parameter of the pore network for 3D bone growth. One thing that is often overlooked is that the bone must be vascularised to survive. If blood vessels do not populate the scaffold, any new tissue forming will die. It is difficult to determine the minimum interconnected size required as it would involve systematic human studies, but early work suggests that a pore diameter of at least 100 μm is required for successful bone ingrowth.[5] Aside from being porous, the scaffolds should be made from a material that is compatible, bonds to bone (is bioactive), stimulates bone growth (osteogenic) and resorbs at a controllable rate. There are several materials that have been designed for implantation that are therefore candidate materials for scaffolds, but very few fulfil all of these criteria.

3. Material selection—why bioactive glass?

One of the most popular materials for repairing bone defects is synthetic hydroxyapatite (sHA, $Ca_{10}(PO_4)_6OH_2$) because it is similar to bone mineral, which is a carbonated hydroxyapatite, and it is bioactive and osteoconductive.[6] Osteoconduction is the growth of bone along a material from the bone/implant interface. Porous forms of hydroxyapatite are commercially available (e.g. ApaPore® (Apatech Ltd., Elstree, UK)), however they have very slow rates of resorbtion are therefore used for bone augmentation (mechanical support of diseased bone), not regeneration. The resorption rates can be increased by creating silicon or carbonate substituted apatites, but the rates are still quite slow.

Alternatives to sHA are bioactive glasses, which may fulfil all of the required criteria for materials selection for a scaffold. They are bioactive and form a bond to bone faster than other bioactive ceramics.[7] This occurs because they form a carbonated apatite layer on their surface, when they are in a physiological fluid, which is very similar to the apatite in bone.[8,9] They have the ability to stimulate new bone growth as they dissolve in the body and are termed osteoinductive. Their osteogenic behaviour is thought to be due to the release of critical concentrations of active ions that stimulate the genes with osteogenic cells.[10,11] The first bioactive glass launched the field of bioactive ceramics and was developed by Hench in 1971.[8] It was made by the conventional melt-derived process, with the composition 46.1 mol% SiO_2, 24.4 mol% Na_2O, 26.9 mol% CaO and 2.6 mol% P_2O_5, and was termed Bioglass®. It is commercially available in the form of a particulate under the trade names Perioglas® (periodontal bone filler) and Novabone® (orthopaedic bone filler) from Novabone Products LLC (Alachua, Fl) and as Novamin, additive for toothpaste, from Novamin Technology Inc. (Alachua, Fl). However, this composition cannot be made into a scaffold as it crystallises on sintering, forming a glass-ceramic. Therefore these glasses show excellent bioactive properties, but are not in a scaffold form.

4. Nanoporous glasses by the sol–gel route

More recently, a method of producing scaffolds from bioactive glasses has been developed that avoids the sintering of particles. The method is the foaming of sol–gel derived glasses. The sol–gel process is an alternative to the traditional melt processing. The process begins with a room temperature hydrolysis of alkoxide precursors, which determine the composition of the glass, to create a colloidal solution (sol) of silica based nanoparticles. The nanoparticles then assemble into a silica network by condensation reactions, forming a *gel* of a –Si–O–Si– network.[12] The steps involved in producing a sol–gel monolith are summarised in Fig. 1. The gelling process generally takes 3 days, but it can be accelerated to a few minutes by using hydrofluoric acid (HF). The condensation process leaves water as a by-product, which remains in the pores of the gel. Thermal processes follow. The aging process usually takes place for several hours at elevated temperatures and strengthens the gel.[13] The pore liquid is then removed in the drying stage, leaving small interconnected pores with diameters in the range 1–20 nm.[13] Stabilisation at increased temperatures follows drying, removing surface silanol groups and three membered silica rings from the network, increasing density, strength and hardness, creating a glass.

An advantage of the sol–gel process over the melt-process is that the sol–gel process provides an interconnected nanoporous structure throughout the glass, which provides a specific surface area two orders of magnitude higher than the dense melt-derived glasses. Sol–gel glasses therefore have enhanced resorbability, bioactivity and bone bonding capabilities *in vivo*.[14,15] The nanoporosity is also thought to affect cell response, especially osteoblast response, as they have been shown to behave differently on surfaces with different nanotopography.[16]

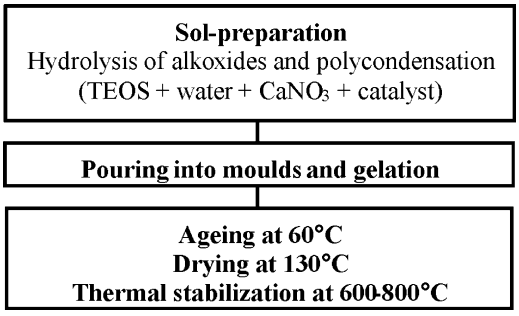

Fig. 1. Flowchart showing sol–gel processing steps.

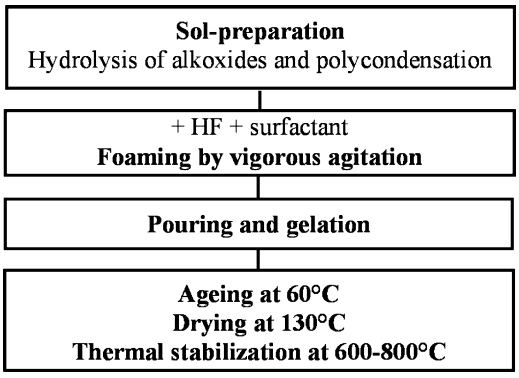

Fig. 2. Flowchart showing the steps for the sol–gel foaming process.

A major advantage of the sol–gel process is that a foaming step can be introduced, which can create porous scaffolds with interconnected macropore networks that fulfil the criteria of an ideal scaffold without crystallising the glass.

5. Sol–gel derived bioactive glass foam scaffolds

When HF is used as a catalyst in the sol–gel process, gelling can occur in a few minutes. This means there is a rapid viscosity increase, which can support a foaming process. A foaming step is added after the hydrolysis step in the original process (Fig. 2).[4,17] The solution is subjected to vigorous agitation in air in the presence of a surfactant. The type and concentration of the surfactant is critical to success[18] as it lowers the surface tension of the liquid, stabilising the air bubbles in the sol. The foam is poured into moulds prior to gelation. The conventional thermal processing involved the sol–gel process is then followed.[17] The macropore network has been shown to be interconnected and to have interconnects in excess of 100 μm (100–300 μm).[19,20]

The resulting tissue engineering scaffold exhibits a *hierarchical* structure with interconnected macropores in the range of 10–600 μm and the nanoporous (1–20 nm) framework inherent to sol–gel glass. The macropore and interconnect size can be tailored by controlling the surfactant concentration and final sintering temperature.[4,18] As these structures are fabricated from bioactive glass, they exhibit the positive attributes of resorbability, bioactivity and biocompatibility.

Fig. 3 shows an X-ray micro-computer tomography (μCT) image of a bioactive glass scaffold. The pore structure is similar to trabecular bone with many interconnects that could facilitate tissue ingrowth.[19,20]

In cell response studies, foamed scaffolds have been shown to stimulate human primary osteoblasts to produce mineralised bone matrix *in vitro* without the addition of growth factors or hormones such as dexamethasone to the cell culture medium, which are needed for mineralization to take place on other materials.[21,22] These results indicate that the scaffolds also fulfil the criterion of being osteogenic, however *in vivo* tests are required to confirm this.

Sol–gel derived bioactive glasses have a very useful surface chemistry as they have a high concentration of silanol groups at the surface. These are involved in the nucleation of the HCA layer. However they are also useful as sites for surface functionalisation.[23] Sol–gel glasses have a particularly high Si–OH content, due to the nature of the process and the nanoporous structure. As-produced glasses are therefore already partly functionalised with OH groups. The final temperature of the sol–gel process can be used to tailor the number of OH groups and the connectivity of the silica network. Sol–gel glasses can then easily be functionalised with groups such as mercapto- and amino-groups[24] that can be used to covalently bond proteins to the surface of the glasses.[25]

Specific nanopore shapes and sizes may also affect which proteins are adsorbed to the glass surface. This can be used in a variety of applications. In vivo, it can be used to deliver proteins or drugs to a defect site at a controlled rate (the rate at which the glass dissolves).[26,27] In vitro, microparticles can be used to as nucleators for protein crystallisation, which enables proteins to be analysed under X-ray diffraction.[28] The shape and size distribution of nanopores (2–30 nm) is thought to be ideal for the immobilisation of proteins. Further heating of the glasses at 800 °C (sintering) can finely control the nanopore diameters.[4]

A criterion that has not yet been discussed is the mechanical properties of the scaffolds. Tailoring the nanoporosity can optimise the mechanical properties of the bioactive glass scaffolds. Scaffolds sintered for 2 h at 800 °C has yielded compression strengths of 2.4 MPa, while maintaining a suitable interconnected macroporous network, which is in the range of the compression strength of cancellous bone.[4] They therefore can match the criterion in terms of compressive strength, so they may be suitable for certain defect sites, such as the Hills-Sacks shoulder defect, but many bone defect sites will be under cyclic loading and as the scaffolds are made from porous glass they are inherently brittle and have poor tensile strength. Toughness must be introduced into scaffolds, which can be achieved

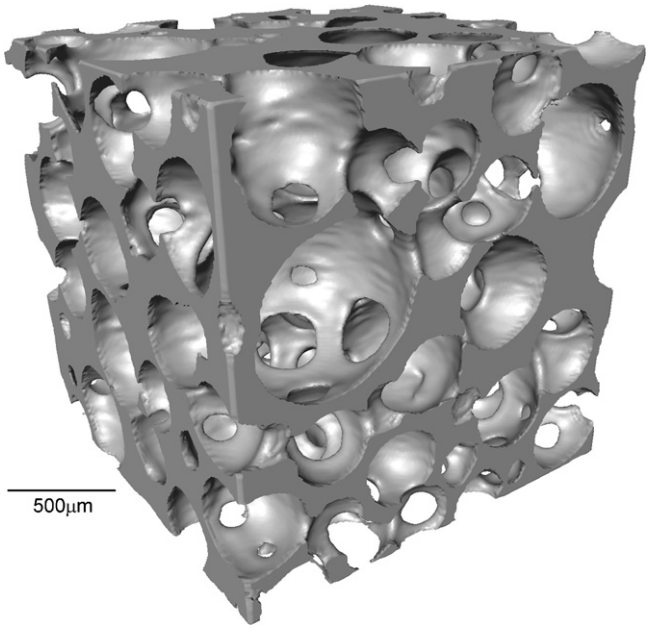

Fig. 3. μCT image of a bioactive glass foam scaffold. Courtesy of Gowsihan Poologasundarampillai, Imperial College London.

by producing composites. An ideal K_{IC} value (a measure of toughness) would be $\sim 3\,MPa\,m^{1/2}$. An obvious strategy for improving toughness is to create a composite.

6. Bioactive composites

There have been several attempts to combine bioactive glasses with biodegradable polymers to create a scaffold material with degradability, bioactivity and toughness.

These attempts commonly used biodegradable polymers that are already approved for clinical use for some applications such as the polyesters polylactides or polyglycolides (and their co-polymers). Composite scaffolds with ideal interconnected macropore networks have been produced by introducing bioactive glass particles into polylactide foams.[29] However, their application in bone regeneration is flawed as the bioactive particles are generally covered by the polymer matrix. The host bone will therefore not come into contact with the glass. Conventional bioactivity tests, where samples are immersed in a simulated body fluid (SBF), showed that HCA formation only occurred in localised regions. A confluent HCA layer took over 21 days to form which suggests bonding to bone tissue would be slow *in vivo*. This may be rectified as the polymer phase begins to degrade and the glass is exposed. However, it is not as simple as that due to how the polymer degrades. These polymers degrade by hydrolysis (chain scission by reaction with water).[30] Initially, degradation is slow and dependent on the rate of water uptake (diffusion) into the polymer. Once the chain scission begins, molecular weight will start to drop. The pH will then drop locally due to the acidic degradation products (e.g. lactic or glycolic acid), which will catalyse the degradation process (self catalysis). This will make the degradation rate to be extremely rapid, causing the scaffold to break down, to rapidly lose mechanical strength, and to cause the bioactive particles to left free to float around the body. The autocatalysis can even cause thicker sections of polymer to degrade faster than thin sections. Co-polymerisation of polylactides with polyglycolides can help tailor the degradation rate of the polymers, but the degradation rate will not be linear. Coupling agents are required between the glass and the polymer. Nanoscale composites may allow a closer relationship between the glass and the polymer and overcome this problem.

7. Future trends: sol–gel derived bioactive nanocomposites

As no current materials fulfil all of the criteria for an ideal scaffold for all bone regeneration applications, new materials must be developed. An ideal scaffold should have the mechanical properties of a conventional bioactive glass/polymer scaffold but have linear (or close to) tailorable degradation rate that has the potential to be matched to the rate of bone growth. The scaffold should also degrade as one material rather than having mismatched degradation rates of a glass and polymer phase such that will leave unresorbed bioactive glass particles after the polymer has degraded.

The aim of creating nanocomposites is to have a nanoscale interaction between the bioactive inorganic phase and the organic phase, creating a tough material. This intimate interaction should allow bone cells to come into contact with both phases at one time, and the material should degrade at a single rate, in a more linear fashion than conventional polyesters or their composites. Nanocomposites can be divided into two classes. One is a nanoscale version of a conventional composite, where nanoparticles are dispersed in a polymer matrix. The second is where the inorganic and organic phases are covalently bonded together at a molecular scale during processing. These materials are termed *hybrids* here, having also previously been termed creamers and ormosils, and have the greatest potential of combining the desired properties of the constituent materials for bone regeneration.

7.1. Nanocomposites by the sol–gel process

Recently, the nanoscale interaction of composite constituents has been demonstrated through the use of sol–gel synthesis techniques.[31,32] The sol–gel foaming process yielded ideal pore networks for bioactive glass scaffolds, so a logical step is to introduce a polymer phase into this process. The aim is to introduce polymer chains into the sol while the inorganic chains are forming, so that the polymer network forms at the same time as the silica based nanoparticles assemble. However, there are complex chemistry challenges associated with this procedure. These include:

- Choice of polymer.
- Calcium precursor.
- Removal of toxic by-products.

Many bioresorbable polymers cannot be simply introduced into the sol due to solubility issues. Calcium is an important component of the materials and must be released with soluble silica to stimulate osteoprogenitor cells. Traditionally calcium nitrate has been used as a precursor and donator of calcium into the inorganic network. However, temperatures of at least 600 °C are needed to drive off the nitrate by-products that are toxic to cells. Nanocomposites cannot be heated to high temperatures as the polymer phase will be damaged. Therefore another calcium precursor is needed. Once all these factors have changed, the foaming process will also need to be modified and any residual chemicals such as surfactants, precursors and catalysts must be removed. How these challenges are being tackled will now be reviewed.

As a proof of principle study, bioactive glass/poly(vinyl alcohol) (PVA) nanocomposite scaffolds were produced using the sol–gel foaming technique.[33,34] PVA was chosen because it is soluble in water and could be added to a typical sol used to synthesise bioactive glass. Up to 30 wt.% polymer was incorporated. The scaffolds produced had pore networks very similar to the bioactive glass foams with macropore diameters of up to 500 μm. Compression testing on these foams demonstrated that polymer addition increased with strain to failure of up to 8% strain, compared to ∼1% without the polymer added. This

is not high enough for an ideal scaffold and is due to the low molecular weight of the polymer used (16,000). This is the maximum molecular weight that can be used for PVA, as it cannot be broken down by the body and a molecular weight of 16,000 is the maximum that can be passed by the kidneys. Potential further improvements could be achieved through changing the polymer. An ideal nanocomposite is likely to contain a degradable polymer with an initial molecular weight in excess of 100 000.

Another problem with the silica/PVA nanocomposites is that the PVA was not covalently bonded to the inorganic phase, therefore the scaffold is likely to break up quite rapidly in body fluid. The calcium precursor used in this study was calcium chloride. It is not yet clear as to whether it was successful in introducing calcium into the inorganic phase.

Bidegradable synthetic polymers that have been approved for clinical use, such as polylactides, polyglycolides and polycaprolactone are difficult to introduce into the sol–gel process as they are insoluble in aqueous solutions and are difficult to couple to bioactive glasses.

An alternative is to opt for natural polymers. An obvious candidate is collagen, a structural protein that provides the tensile strength and toughness of many tissues, including bone. It has not yet been incorporated into the sol–gel process but it has been mixed with novel bioactive glass nanofibres to create a nanocomposite.[35] The bioactive glass phase was produced by electrospinning of sol–gel derived bioactive glass. The glass nanofibres were ~320 nm diameter. Solubilised collagen was added to a phosphate buffered solution (PBS) to induce fibril reconstitution resulting in a suspension of collagen fibrils. Separately, a suspension of the bioactive glass nanofibres was prepared in PBS. The two suspensions were mixed and freeze-dried. Cross-linking was induced through a chemical means. The scaffolds produced via this method had a suitable interconnected macroporous structure with pores ranging between tens and hundreds of microns.[35] However, the method is likely to crosslink the collagen phase only, without any covalent bonds forming between the collagen and the glass. *In vitro* studies were carried out on the scaffold demonstrating apatite layer formation in SBF and favourable proliferation of osteoblast cells on the bioactive glass containing scaffold compared with collagen alone. At the time of writing, no details of the composite's mechanical properties were available.

All of these nanocomposites suffer from a common problem in that there is no covalent bonding between the inorganic and organic phases. This is a common problem even in conventional composites.

7.2. Inorganic/organic hybrid nanoscale composite scaffolds

Although conventional polyesters are insoluble in water, they can be functionalised so that not only are they incorporated in the sol–gel process, but they can form covalent bonds with the silica network, creating a true hybrid material. The functionalisation of the polymer involves the introduction of coupling agents. This is the real future of bioactive composite scaffolds.

One example is the synthesis of silica/poly(ε-caprolactone) (PCL) hybrid discs.[31,36–38] Hydroxyl groups at either end of the poly(ε-caprolactone diol) polymer chains were reacted with 3-isocyanatopropyl triethoxysilane (IPTS). This resulted in a polymer end capped with a triethoxysilyl group. The end capped PCL was introduced into a sol to yield an interconnected PCL-silica network. The hybrid showed bioactivity after 1 week in SBF.[39] The formation of the HCA layer occurred even though there was no calcium in the composition. This is likely to be due to the high calcium concentration in the SBF and a high concentration of Si–OH groups within the silica network. Calcium must be incorporated for *in vivo* osteogenesis.

The mechanical properties of the bioactive glass/PCL hybrid with 60 wt.% polymer showed promising results, having a Young's modulus and tensile strength of 600 and 200 MPa, respectively,[39] which is in the range of cancellous bone. However, the mechanical properties were measured on dense materials and would be dramatically lower if the materials were processed into porous scaffolds. The molecular weight of the PCL was low, at 6693, indicating that long-term mechanical properties and stability in body fluid may also be low. Polymer chains must entangle with each other if the material is to have toughness, and the molecular weight should be two orders of magnitude higher for entanglement to happen.

Natural polymers can also be functionalised. One example is chitosan (2-amino-2-deoxy-2-Ducan), which is derived from crustacean shells. The chitosan was reacted with methanesulphonic acid to form butyrylchitosan, which was then reacted with acryloxypropyl trimethoxysilane (APTMS) to form a silanated butyrylchitosan, which was then introduced into a sol of hydrolysed TEOS.[40] Currently, only thin films have been produced. An alternative natural polymer is gelatin, which consists of polypeptide fragments derived from collagen. GPTMS has been used to functionalise the gelatin molecules for incorporation into the sol.[41,42] Porous scaffolds have been produced with open porosity by soaking the wet gels in ammonia, freeze drying them (freezing followed by sublimation of ice crystals). No mechanical data is yet available (freeze dried materials are usually weak) but the hybrids showed biocompatibility and osteogenic properties with MC3T3-C (mouse originated osteoblasts) cells.[43] The scaffolds contained calcium, but in the form of calcium nitrate, which could cause long-term toxicity. An alternative is therefore needed to the calcium nitrate precursor. Calcium chloride has been used. But an interesting alternative comes in the form of star gels.

'Star gels' are a type of organic–inorganic hybrids that have an organic core surrounded by flexible arms, which are terminated in alkoxysilane groups. These groups then form a silica-like network through the sol–gel process. Their mechanical properties lie between conventional glasses and elastic polymers.[44,45] Star gels are synthesised through the hydrolysis and polycondensation of a specific single component inorganic–organic precursor.

Manzano et al.[45] developed the first bioactive version of a star gel hybrid by incorporating calcium methoxy ethoxide into the sol. Monoliths (not macroporous) were shown to have a Young's modulus and compressive strength of 1 GPa and

250 MPa, respectively, comparable to that of human bones. The fracture toughness of the material was measured at ~3 MPa $m^{1/2}$, which is in the range of cortical bone. This was three times higher than a conventional sol–gel bioactive glass that was used in comparison. Also under cyclic fatigue tests the star gel outperformed a human femur by twice the number of cycles to failure. The resorption characteristics and cytotoxicity were not considered.

8. Conclusions

As yet, the design criteria for an ideal scaffold for bone regeneration have not been fulfilled. Sol–gel derived bioactive glasses have the osteogenic properties required and they have a nanoporosity that provides controlled degradation and sites for cell attachment and protein adsorption. Foaming these materials produced the first porous bioactive glass scaffolds with interconnectivity suitable for vascularised bone ingrowth and a compressive strength similar to cancellous bone. Functionalised biodegradable polymers can be introduced to the sol–gel process, creating inorganic/organic hybrid nanoscale composites. The intimate interaction between the inorganic and organic chains has the potential to combine bone bonding and bioactive ion release with toughness and controlled degradation. However, the chemistry and materials processing routes are complex, so the ideal materials are yet to be developed. The materials must be optimised from the atomic level (connectivity of the silica network and polymer functionalisation) through the nano (distribution of chains and number of cross-links and nanoporosity) through to the macroscale (interconnected pore network). All these factors must be optimised with respect to degradation rate, mechanical properties as a function of time in body fluid and cellular response. Achieving this will involve close collaboration between materials scientists, synthetic chemists, cell and molecular biologists, physicists and orthopaedic surgeons.

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