Phosphate Based Glasses: A Perspective

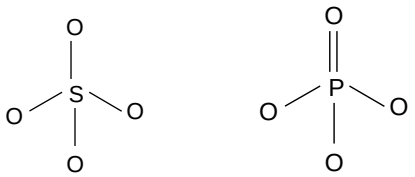
Ensanya Ali Abou Neel[*a], David Mark Pickup[b], Sabeel Padinhara Valappil[a], Robert John Newport[b] and Jonathan Campbell Knowles[+a]

The general trend in biomaterials is to use and employ materials that play an active role in tissue regeneration rather than passive and inert materials. Therefore, understanding how a material interacts with the surrounding environments, including cells and tissue fluid, allows material design to be tailored so that implants can be constructed to promote a specific biological response, helping them better perform their function. This class of materials has been described as the "Third Generation" of biomaterials. Phosphate based glasses fall into this category and it has been shown that the properties of these glasses can be tuned via their composition according to the desired end application. These glasses can be prepared as melt quenched or sol-gel bulk form suitable for potential hard tissue engineering applications and as vehicles for antimicrobial agents. They can also be prepared as fibres suitable for soft tissue engineering applications such as those involving muscle, ligaments, and tendon, where, like the fibres, the tissue has a high degree of anisotropy.

1. Introduction

Recently, interest in soft and hard tissue engineering for improved tissue regeneration has fuelled the need for novel biodegradable materials having a specific and controllable bioactivity [1]. Bioactive glasses, silicate based in particular, have been the subject of intense interest for the last three decades as materials for tissue regeneration applications. In vivo, when they exposed to physiological fluids, they form a surface apatite layer; this layer has the capacity to bond to collagen synthesised by connective tissue cells such as osteoblasts [2]. One commercially available bioactive glass is Bioglass® which has a composition known as 45S5 corresponding to 45.0 wt% SiO_2, 24.5 wt% CaO, 24.5 wt% Na_2O and 6.0 wt% P_2O_5 [3-5]. Today, the 45S5 composition is used as a benchmark by which the performance of new silicate based bioactive glasses is measured. Such glasses have shown great success in many clinical applications especially in dental and orthopaedic fields. However, there are questions raised related to the long term effect of silica [6] and the slow degradation of these glasses, often taking 1 to 2 years to disappear from the body [7, 8]. Because of these limitations, the search for new materials for the repair of bone defects has continued and has led to the emergence of phosphate based glasses as potential alternatives.

Phosphate based glasses in the $CaO\text{-}Na_2O\text{-}P_2O_5$ system have unique dissolution properties in aqueous based fluids. Degradation rates can be varied from hours to several weeks by changing the glass composition. Moreover, all the constituents of these glasses are elements naturally inside the body and therefore can be excreted by the normal physiological processes. Furthermore, these glasses can be synthesised to include dopants that are able to induce a specific biological function and enhance biocompatibility [9-12].



(a) (b)

Figure 1: Silicate (a) and phosphate (b) tetrahedra.

This review starts with a general description of phosphate glasses, highlighting their differences with their silicate based counterparts and explores what these glasses can offer in terms of biomedical applications. Particular focus is placed on phosphate glass chemistry, terminology and structure. Next we discuss the processing techniques used to prepare these glasses. In the case of the melt-quenched prepared glasses, both monoliths and glass fibres are described. Recent developments in using sol-gel methods to prepare phosphate glasses for biomedical applications are reviewed. The article concludes with a discussion of the future of phosphate based glasses as biomaterials and highlights possible avenues of potential application.

2. Comparison of Phosphate and Silicate Based Glasses

Silica (SiO_2) is a classic network forming oxide. It is common in nature in both crystalline and glassy forms because of the strong affinity of silicon towards oxygen, and the natural abundance of these two elements. The basic unit of the silicate based glasses is the SiO4 tetrahedron shown in Figure (1a). This unit can share up to four oxygen atoms with other such tetrahedral units to form a 3D network structure.

Phosphorus also has an affinity towards oxygen and as a

consequence phosphates are common in nature. In common with silicate based glasses, the building block of phosphate glasses is a tetrahedral unit. However, as can be seen in Figure 1, the PO_4 unit is quite different from the SiO_4 unit. This is because phosphorus nominally has a charge of 5+ whereas silicon has a charge of 4+. Therefore when SiO_4 tetrahedra form a network they can share all four of their oxygen atoms to give the stoichiometry $SiO_{4/2}$ or SiO_2 which is charge balanced (assuming a charge of 2- on the oxygen). In contrast, when forming an analogous charge balanced 3D binary oxide, phosphorus can only share three out of its four oxygens which gives the stoichiometry of $PO_{(1+3/2)}$ or P_2O_5. In the case of the P_2O_5, the oxygen atoms that are not shared between phosphate tetrahedral share their two unpaired electrons with the P5+ ions to form a terminal double bond (Figure 1b) [13].

The fact that phosphate anions contain at least one terminal oxygen limits the connectivity of phosphate based glasses relative to their silicate based counterparts. Therefore, in general the rigidity, which is related to the interatomic forces, is less in phosphate glasses compared to silicate glasses. Moreover, when mixed with metal oxides, phosphate glasses contains fewer cross-links but a higher number of terminal oxygen atoms than silica glasses of the same metal oxide content. These two structural properties result in more flexibility in the orientation of PO_4 tetrahedra [13]. Therefore, the range of glass formation in binary phosphate glass in systems is wider than in the analogous silicate based system, even in the presence of alkali or alkali earths.

Pure vitreous silica is thermally and chemically stable. The addition of modifying oxides such as Na_2O, K_2O, MgO, and CaO, which are not part of the glass network and disrupt it resulting in terminal Si–O bonds, produces less stable glasses [14-15]. Pure vitreous phosphorus pentoxide (P_2O_5), on the other hand, is chemically unstable with regard to hydrolysis of the P–O–P bonding by atmospheric moisture; in this case the addition of metal oxides improves its stability because P–O-Mn+ (where M = metal cation) bonds are generally more stable towards atmospheric hydrolysis [16].

From a technical standpoint of pure melt quenched, vitreous silica is only used in very specialized applications due to the very high melting temperatures involved in its manufacture. The addition of modifying oxides which reduce network connectivity significantly reduce the melting temperature required. Phosphate based glasses, however, can be prepared at relatively low temperatures [6].

These two types of glass also differ in their aqueous dissolution mechanisms and in the stability of resultant anionic species. Dissolved silicate species can easily be polymerised or repolymerised to form species with no resemblance to the original glass structure, whereas phosphate chains and rings are quite stable in aqueous solution [17].

Due to their high melting temperatures, silica based bioactive glasses are difficult to draw into fibers and the addition of metal oxides such as Na_2O and CaO to lower the melting temperature can adversely affect the glass bioactivity by increasing its tendency towards crystallization. The of use sol-gel chemistry to overcome the problems of fiber drawing

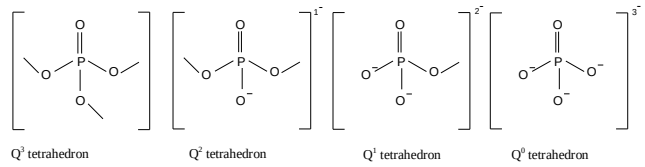


Figure 2: Nomenclature and representation of PO_4 tetrahedra with different polymerizations [22].

is still in its infancy [18]. In contrast, phosphate glasses containing more than 45 mol % P_2O_5 can easily be drawn into fibers [10]. From a structural viewpoint, the fibre drawing ability or spinnability is related to the ability of the longer phosphate chains to entangle with other chains. Such interactions allow continuous filaments to be formed instead of clusters or droplets. Milberg and Daly [19] proposed that in perfectly oriented fibres, all chain axes are parallel to the fibre axis, and the rotational disorder of the chains around their own axes corresponds to the cylindrical symmetry of the fibre. Moreover, Murgatroyd [20] suggested that the drawing operation preferentially selects the strong P-O-P bonds and the continuity of fibres is related to the ability of these bonds to be aligned along the long axis of the fibre. Whereas, weak P-O-P bonds can be extended for a short distance along the strong bonds, but are not able to form continuous fibres [20].

Compared to silicate glasses, phosphate glasses have poor chemical durability and the poor durability limits their use in technological applications. However, phosphate glasses are preferred in other applications such as release of oligo-elements in soils. Moreover the solubility of phosphate species in Bioglass is responsible for the nucleation and apatite layer formation that is considered to be the main factor responsible for the bioactivity of Bioglass [21].

3. Terminology and Chemistry of Phosphate-Based Glasses

As discussed in the previous section, the PO_4^{3-} tetrahedron is the basic building block of structure the phosphate based glasses. Phosphate tetrahedra are classified by the number of oxygen atoms they share with other phosphate tetrahedra. An oxygen atom shared in this way is usually referred to as bridging oxygen, abbreviated to BO hereafter. The various types of phosphate tetrahedra that result from this classification are labeled using Q^i, where i refers to the number of BOs and ranges from 0 to 3. For example, Q^3 PO_{43-} tetrahedra share three covalently bonded BOs with neighbouring PO43-tetrahedra as found in vitreous P_2O_5. In terms of the structure of the phosphate network, Q^3 moieties are known as a branching units and since they have a O/P ratio of 2.5 (i.e. $PO_{(1+3/2))}$ they have neutral charge. Q^2 tetrahedra possess two BOs which results in an O/P ratio of 3 and a net charge of 1-. Structurally, Q^2 units can be considered as PO_3^- middle groups in phosphate chains. Q^2 tetrahedra have one BO and hence an O/P ratio of 3.5 and a net charge of 2-. Structurally Q^1 units can be considered as representing $P_2O_7^{4-}$ dimmers or as terminating groups at the end of phosphate chains. Q^0 represents an isolated $(PO_4)^{3-}$ tetrahedron (i.e. O/P = 4) with no BOs to neighbouring tetrahedra; $(PO_4)^{3-}$ groups are also known as orthophosphate

units [22-24]. The various Q species discussed above are illustrated in Figure 2.

The structure of vitreous P_2O_5 consists of only Q^3 phosphate tetrahedra that form a three dimensional network; the addition of modifying metal oxides, however, results in the "depolymerisation" of this network via the cleavage of P-O-P bonds with the creation of negatively charged NBOs at the expense of BOs. The negatively charged NBOs charge balance the metal cations and coordinate them such that they achieve their preferred coordination number [13, 23, 25, 26]. The depolymerisation model proposed by Kirkpatrick and Brow [23] predicts that the dominant Qi species changes according to $Q^3 \rightarrow Q^2 \rightarrow Q^1 \rightarrow Q^0$ as the amount of modifying oxide increases. Increasing the amount of metal oxide results in an increase in the overall O/P ratio and this determines the average number of BO per phosphate tetrahedron; from this one can predict the dominant Q^i species.

3.1. Ultraphosphate Glass Structure

Ultraphosphate glasses are those with compositions in the range $(M_{2/v}O)x(P_2O_5)1\text{-}x$, where $0 \leq x \leq 0.5$ and v is the valance of the metal cation. The ultraphosphate composition can also be described in terms of the atomic ratio of oxygen phosphorus, i.e. $3.0 \leq O/P \leq 2.5$. Glasses with compositions in this range are expected to have network structures dominated by Q^2 and Q^3 species. Structural studies using 31P NMR have confirmed this to be the case and verified that the concentrations of Q^2 and Q^3 groups can be calculated from Van Wazer's chemically simple depolymerisation model [27]. The Q^2 and Q^3 tetrahedra appear to be randomly linked, at least in alkali ultraphosphate glasses. In glasses with a high P_2O_5 concentration (greater than 75-80 mol%), the phosphate network resembles that of vitreous P_2O_5. With increasing modifier content, there is a loss of the extended-range order associated with vitreous P_2O_5, as the concentration of Q^2 species. The composition at which this transition occurs depends on the preferred coordination number and valence of the modifying cation since it relates to the relative concentrations of NBOs and cations. Hoppe [13] has postulated that at low cation concentrations, both NBOs of the Q^2 units coordinate to the same cation; whereas at higher cation concentrations, each NBO can coordinate a separate cation resulting in a structural relaxation.

3.2. Metaphosphate Glass Structure

Metaphosphate glass have the composition $(M_{2/v}O)0.5(P_2O_5)0.5$ and an O/P ratio of 3. Their structures consist of infinitely long chains and/or rings of Q^2 units [24]. These phosphate chains are linked through bonds between the terminal NBOs and the modifying cations. Structural studies have revealed that the properties of metaphosphate glasses are less dependant on the nature of the P–O–P that form the chains than on the P–O–Me bonding between chains. In general, as the field strength of the modifying cation increases, there is an increase in the rigidity of the metaphosphate network and an associated increase in glass transition temperature [23].

3.3. Polyphosphate Glass Structure

Polyphosphate glasses have a composition compositions in the range $(M_{2/v}O)x(P_2O_5)1\text{-}x$, where $x > 0.5$, and an $O/P > 3$. The structure of polyphosphate glasses is based on Q^2 chains terminated by Q^1 units. At the pyrophosphate composition ($x = 0.667$), the structure is dominated by phosphate dimers, i.e. two Q^1 units sharing a bridging oxygen atom. At $x = 0.75$, only isolated orthophosphate Q^0 units are present [24]. Polyphosphate glasses are often more durable than their metaphosphate counterparts due to the reduction of the more readily hydrolyzed Q^2 units in the structure [23].

4. Melt Quenched Phosphate Based Bulk Glasses

Most phosphate based glasses are prepared by melt-quenching methods. A mixture of oxide precursors is melted in a furnace at temperatures of over 1000°C; the exact temperature used depends on the final composition of the glass. Once a homogeneous melt has been achieved, the glass is formed by casting different shapes such as rods and plates. To remove residual stress, the melts are normally cooled quickly through the glass transition temperature (Tg) and then the cooled very slowly to room temperature in an annealing step.

4.1. Technological Applications

Phosphate based glasses have been used in a wide range of technological applications such as sensors, solid-state batteries, laser devices, and air tight seals for metals with a high coefficient of thermal expansion [28]. Phosphate glasses have also been developed for achromatic optical elements due to their low dispersion and relatively high refractive indices. Iron-containing phosphate glasses have found uses as matrices for vitrifying nuclear waste products [29-31]. Their capacity for high waste loading, low processing temperature and high chemical durability offer significant advantages compared to most silicate and borosilicate glasses. They are reported to maintain their high chemical durability even after devitrification of waste forms [32].

4.2. Medical Applications

4.2.1Controlled Release Glasses (CRG)

Controlled release glasses are a class of materials that completely dissolve in aqueous media leaving no solid residues. Their degradation is an erosion controlled process that follows zero order release over the life of the material, i.e. the release rate is constant and independent of time and concentration [33]. They can be produced in different forms such as powder, granules, fibre, cloth, tubes, and monoliths of various shapes [34]. These glasses have been under development in the Standard Telecomminucation Laboratories since the early 1970s [35]. They find application in many different areas such as feeding of bacteria, controlling parasite infection in water canals, veterinary use or even treatment of infections in humans. Some examples of these uses are described below.

Polyphosphate glass provides a source of phosphate ions that can support the growth of recombinant Escherichia Coli to a density 40 % higher than that obtained with typical

fermentation media. The high solubility of polyphosphates together with the absence of precipitate formation when mixed with the fermentation media are key benefits for such applications [36].

Soluble phosphate glasses containing such as copper, cobalt, and selenium, designed for oral administration in form of a rumen bolus to ruminant animals for the treatment of trace element deficiencies, are manufactured under the trade name of Cosecure® [37-40].

Copper releasing phosphate glasses have been used as molluscicides to control the snail hosts of schistosomiasis. Glass composition and physical form can be tailored in a reproducible manner to suit the chemistry of the water body being treated. Moreover, most of the released Cu is in non-toxic or weakly toxic forms such as copper polyphosphate complexes which acts as secondary releasing agents [38-40].

Silver releasing phosphate glasses are used clinically to combat long term infection in indwelling catheters. A cartridge with silver containing glass is inserted in line between the catheter and urine collection bag. This cartridge treats the urine as it flows through it from the bladder to the collection bag. The silver ions released are found to inhibit bacterial proliferation [34] and have the potential to be used in the treatment of vesicoureteral flux and urinary incontinence [41].

4.2. 2. Hard Tissue Engineering Applications

Biodegradable scaffolds, which are eventually replaced by the natural tissue, are desirable constructs for tissue engineering applications. CaO-Na_2O-P_2O_5 glasses have properties that lend them to use as hard tissue substitutes or as substrates for synthetic orthopaedic graft materials. Compositionally they are similar to the inorganic component of bone [42]. Furthermore, fluoride-doped phosphate glasses have been developed that play an active role in stabilising the apatite layer [42]. Phosphate glasses can also be doped with a variety of metal oxides to modify their physical properties [43, 44]. It is reported that the ionic environment caused by the leaching of ions from these glasses during their degradation has an impact on the biological response of cells [45]. For example, Ca^{2+} ions have been implicated in stimulating osteoblast-like cell proliferation and differentiation and phosphate ions act as extracellular 'pool' responsible for the release of Cbfa-1, an important bone marker, from bone cells [45].

For hard tissue engineering applications, a number of glass systems have been developed by additions of various metal oxides such as Fe_2O_3, Al_2O_3, ZnO, and TiO_2 into the parent glass. Comprehensive studies have been reported that give an overview of the correlation between the basic glass structure and the biocompatibility.

4.2.2.1. Binary System (s)

Binary sodium phosphate glasses (Na_2PO_4H-$NaPO_4H_2$), developed by Gough et al. [46, 47], demonstrated a minimal level of macrophage activation evident from low amounts of peroxide and interleukin-1β release. Moreover, early primary craniofacial osteoblast attachment and spreading was also observed on those glasses. Upon long term culture (28 days), the craniofacial osteoblasts exhibited cytoskeletal characteristics and a level of collagen synthesis similar to those of the positive control. The biocompatibility of these glasses was related to their degradation and the ions released. However, it was difficult for cells to attach to such highly soluble glasses because the labile surface prevented the formation of a physical anchorage.

4.2.2.2. Ternary System (s)

Uo et al. [48] developed the P_2O_5-CaO-Na_2O ternary glass system and assessed the cytocompatibility by direct contact cytotoxicity assay using dental pulp cells. Their study reported that the samples containing 50 mol% P_2O_5 were not very cytotoxic. The cytotoxicity was found to decrease with increasing P_2O_5 content due to a change in pH from neutral at 50 mol % to acidic at 60 mol% or more. Thus, the authors related the cytotoxicity to the glass degradation, the associated pH changes and ionic concentration in the media.

Franks et al. [49] studied the same ternary glass system in the composition range $(P_2O_5)45(CaO)x(Na_2O)55\text{-}x$, where x was between 8 and 40 mol %. Initial work focused on the degradation and ion release of these glasses; the results suggested that the interaction of the Ca2+ ions with the glass network controlled glass degradation, and an inverse relationship existed between calcium oxide content and the degradation rate. The biological response of this glass system was tested by Salih et al. [6] to assess their suitability for potential bone regeneration applications. Two human osteoblast cell lines, MG63 and HOS (TE85) were incubated in the glass extracts with different concentrations (neat, 1:4, 1:16, 1:64 dilution) for two and five days. MTT assay was used to study cell growth, and ELISA was used to measure the expression of antigens such as bone sialoprotein, osteonectin, and fibronectin which play a vital role in bone metabolism and integrity. The results showed that the glasses with lower solubility enhanced bone cell growth and antigen expression at all tested dilutions. The highly soluble glasses, however, significantly reduced cell proliferation, and down-regulated antigen expression especially with neat and 1:4 dilutions at five days. The authors suggested that these results were related to ions released from the glass during degradation and the resultant pH changes. They also suggested that with less soluble glasses, greater amounts of Ca^{2+} ions are released, which is known to have an essential role in cell activation mechanisms affecting both cell growth and function. However, with highly soluble glasses, a sharp increase in pH associated with high release rates of Na^+ and phosphate ions (PO_4^{3-}) may have a deleterious effect on cells.

Due to the high degradation rate and unfavorable cellular response associated with high sodium content glasses, Franks studied the effect of replacing the Na_2O with K_2O [42]. For this new study, glasses of composition $(P_2O_5)45(CaO)x(K_2O)55\text{-}x$, where x is between 16 and 32 mol % were used. Glass with CaO content outside this range was difficult to prepare due to its crystallisation upon casting. It was observed that the P_2O_5-CaO-K_2O system dissolved at a higher rate than the analogous P_2O_5-CaO-Na_2O system and hence no biocompatibility study was conducted on this glass system.

Bitar et al. [50] investigated the short-term response on

exposure to phosphate based glass of two typical cellular components of a hard/soft tissue interface, periodontal ligament/mandible and patellar tendon/tibia. Human oral osteoblasts, oral fibroblasts and hand flexor tendon fibroblasts were co-cultured on glasses with different degradation rates ($(P_2O_5)45(CaO)x(Na_2O)55-x$ where x = 30-48). Quantitative and morphological assessment of cell adhesion and proliferation for all cell types was recorded. Immunolabelling was also used to establish phenotypying of both osteoblasts and fibroblasts. The results showed that glass discs with less than 40 mol% CaO support little or no cell adhesion and survival. This behaviour was related to the high solubility of the surface layer of these glasses; therefore, it is difficult for cells to attach to a labile surface and to form a physical anchorage as observed by Gough et al., [46, 47]. The authors concluded that ternary glass compositions with high CaO content (46 and 48 mol%) support high numbers of adherent and viable cells as indicated by DNA content, and also maintain cellular function as indicated by phenotypic gene expression up to 7 days.

From these studies, it is clear that the glass degradation, and the associated ion release, and pH change of the surrounding environments, are factors affecting biocompatibility. Therefore, additions of metal oxides known to affect the chemical durability of phosphate glass to the P_2O_5-CaO-Na_2O system are expected to have an effect on biocompatibility. The quaternary systems that result from such additions are discussed in the next section.

4.2.2.3. Quaternary Systems/Dopants

Knowles et al., [51] synthesized quaternary glasses of composition $(P_2O_5)45(CaO)x(Na_2O)55-x-y(K_2O)y$, where y = 20, 24, 28 or 32 and x = 0-25, in order to study the affect of substituting Na^+ ions with larger K+ ions. The aqueous degradation of this system was affected by both CaO and K_2O content. An anomaly in degradation was observed at high CaO content, where weight gain was observed prior to weight loss. The MTT assay showed that the K^+ had a positive effect on cell proliferation only at high content, 20 mol % K_2O, regardless of the associated increase in degradation.

In order to understand the effect of changing the radius of the divalent cation in a quaternary system, Franks et al. [52] substituted Ca2+ ions with smaller Mg^{2+} ions in $(P_2O_5)45(CaO)32-x(Na_2O)23(MgO)x$, where x = 0-22, glasses. This study concentrated on the overall degradation characteristics of the glasses and the effect of released ions on cell proliferation. The results showed that degradation of the glass as a function of time changed from exponential to linear with decreasing CaO content. This emphasised the influential role of CaO on the degradation process. The degradation rate was decreased by substitution of CaO with MgO despite Mg^{2+} having the same valence as Ca^{2+}. The MTT assay was used to assess the effect of different dilutions of glass extracts on the proliferation of human osteoblasts (MG63) for two and five days. The results were normalised to the control cells incubated in normal medium. The result showed that glasses with little or no MgO showed a slight decrease in cell proliferation only after two days; however, after five days all glass compositions tested showed equal or greater cell proliferation than the control.

Salih et al. [53] added zinc oxide to PBG with the aim of promoting osteoblast cell adhesion and improving the potential for use in bone tissue engineering applications. The compositions investigated were $(P_2O_5)50(CaO)40-x(Na_2O)10(ZnO)x$, where x = 0-20. Attachment of osteoblast-like cells was assessed morphologically by scanning electron microscopy and the effect of the glass extract (neat and 10% diluted) on cell proliferation over periods of up to 7 days was determined by cyquant assay. The results showed that after 24 hours of culture, the cells attached to all glass compositions but still maintained round morphology suggesting lack of spreading on the glass surfaces. Moreover, cell proliferation increased with increasing ZnO content up to 5 mol%, but never reached levels exhibited by cells grown on the positive control.

Abou Neel et al. [54, 55] prepared bulk quaternary glasses containing TiO2 by conventional melt quenching methods. The aim was to test the hypothesis that the combination of Ti^{4+} and Ca^{2+} ions would further improve the biological response of phosphate glasses. The glass compositions studied were (P2O5)50(CaO)30(Na2O)20-x(TiO)x, where x = 1, 3 and 5. MG63 cell proliferation, gene expression, and bioactivity were the focus of this study. Cell proliferation and gene expression (Core binding protein factor alpha 1 (Cbfa1), alkaline phosphatase (ALP), Collagen type I alpha subunit I (COLIAI), and Osteonectin (Sparc)) were reproducibly enhanced on the surfaces of the Ti4+containing glasses, particularly those with 3 and 5 mol% TiO_2. The authors suggested that this enhancement may be associated with the lower degradation of these compositions which help maintain pH at a level favoured by osteoblasts. It was also suggested that the release of Ti^{4+} ions may have a beneficial effect on bioactivity.

Of the three compositions of Ti-doped phosphate based glasses investigated, the 5 mol% TiO_2 glass induced the most favourable cellular response [54]. As a follow-up study, Abou Neel et al. [56] replaced some of the CaO with in this glass with ZnO (1, 3 and 5 mol%) in an attempt to further improve the biological properties. This work concentrated of the effect of ZnO additions on the thermal properties, degradation, ion release, surface and biological properties. The results showed that the addition of ZnO was effective in controlling the bulk, and surface properties of the glass. Glasses containing both TiO2 and ZnO demonstrated similar high viability of MG63 cells up to 7 days to both the 5 mol% TiO_2 parent glass with and the positive control, Thermanox®. This cell proliferation was correlated with the release of beneficial Ca2+, P, Ti4+ and Zn^{2+} ions at suitable level coupled with an increase in surface hydrophilicity. The hydrophilicity is thought to be associated with enhanced protein adsorption and adhesion of anchorage dependant cells such as osteoblast, fibroblast and endothelial cells on the surface of biomaterials [57].

4.2.3. Antimicrobial Delivery Devices

Phosphate glasses offer potential alternatives to the current methods available for the treatment of infections since they can be used as localised antibacterial delivery systems via the

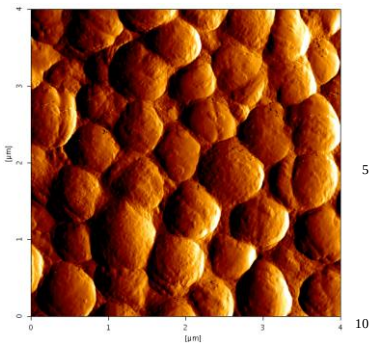


Figure 3: AFM image of silver free glass surface coated with *S. aureus* biofilm.

inclusion of ions known for their antibacterial effects such as copper, silver, and gallium. Such materials could therefore be placed at a site of infection, with the aim of releasing antibacterial ions as the glass degrades, which may be useful in wound healing applications. Their use could be extended to the prevention of implant or biomaterial related infections, which are one of the main causes of revision surgery, and to augment or replace the current prophylaxis of systemically administered antibiotics [58].

Antimicrobial glass systems incorporating either Cu^{2+} or Ag^{+} ions were successfully prepared by Mulligan et al. [59, 60] for potential applications in the treatment of oral infections. The aim was to develop glass devices that could be placed at the site of an infection such as in a periodontal pocket to treat the infection with the antimicrobial ions released as the glass degrades. Both reports focused on glass systems with a fixed P_2O_5 concentration of 45 mol %, and concentrations of the antibacterial ions, Cu^{2+} or Ag^{+}, of 0, 1, 5, 10 and 15 mol %. For each system, the calcium oxide to sodium oxide (CaO/Na_2O) ratio was varied to give the same degradation rate over all compositions. Consequently, the overall effect on bacteria was due to the presence or absence of antibacterial ions and their concentrations. The effect of both glass systems on the viability of a *Streptococcus Sanguis* biofilm using constant depth film fermenter (CDFF) was evaluated in a simulated oral environment using the glass sample containing no antimicrobial and HA (hydroxyapatite) as controls. The results demonstrated that after 24 h, there was a significant reduction in viable counts of bacteria compared to the controls, which was attributed to the release of antimicrobial ions. This reduction was correlated with the concentration of antimicrobial ions in the glass. Despite recovery of the bacterial counts after 48 h, they were significantly lower than those of the controls and remained relatively constant between 48 h and eight days. Two possible reasons were proposed for this recovery: firstly, the formation of a sacrificial layer of dead bacterial cells that acts as a barrier against further penetration of the antimicrobial ions into the biofilm; secondly, the differentiation of bacteria into another phenotype that was resistant. The results also showed that Ag^{+} ions display more potent antimicrobial activity than to Cu^{2+} ions.

Further work on antimicrobial phosphate glasses was carried out by Ahmed et al. [61] who investigated glasses with a relatively higher phosphate content. The compositions

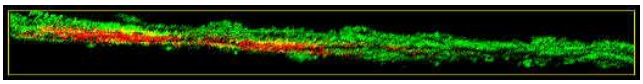


Figure 4: A cross- sectional view of the *S. aureus* biofilm on 20% silver doped PBGs. Viable (green) and non-viable (red) bacteria (62).

studied were $(P_2O_5)50(CaO)30(Na_2O)20\text{-}x(Ag_2O)x$ where x = 0-15. Disc diffusion assay was used to screen the antibacterial activity of these glasses against various micro-organisms including *Staphylococcus aureus* [Figure 3], *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*, methicilline resistant *Staphylococcus aureus*, and *Candida albicans*. The results showed that the phosphate glasses containing 3 and 5 mol% Ag_2O were more effective than the remaining compositions in the inhibition of bacterial growth. Overall it was concluded that the glass with 3 mol% Ag_2O was of optimal composition to mount a potent antibacterial effect against the test micro-organisms since it was bactericidal against *Staphylococcus aureus*, *Escherichia coli*, and significantly reduced the growth of *Candida albicans*. These findings were correlated with the excellent long term release of Ag ions from that composition into the surrounding medium.

Further study of silver containing phosphate glasses (10, 15 and 20 mol%) was performed by Valappil et al. [62] who tested their effect on the formation of the highly resistant *S. aureus* biofilms. Silver ions were found to reduce the growth of *S. aureus* biofilms. Variations in bactericidal activity were correlated with glass degradation rates which varied between 0.42 and1.22 $\mu g.mm^{-2}.\ h^{-1}$ depending on composition. Due to the antibacterial action of the Ag^{+} ions, a dead layer, approximately 20µm thick, of non-viable bacterial cells was formed on the glass surface [Figure 4]; this layer was covered by a top layer of viable cells. The antibacterial effect of these glasses was attributed to the silver ions being present in the most potent +1 oxidation state; confirmation of this was provided by Ag K-edge XANES (X-ray absorption near-edge structure) measurements.

As well as silver and copper, gallium was also investigated as a dopant for phosphate glasses because of its antibacterial activity [63]. Novel quaternary gallium-doped phosphate based glasses $((P_2O_5)45(CaO)16(Na_2O)39\text{-}x(Ga_2O_3)x$ where x = 1, 3 and 5) were synthesized, and their bactericidal activities tested against both Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram positive (*Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, and *Clostridium difficile*) bacteria. The report confirmed that the controlled delivery of Ga^{3+} ions from the glass containing 1 mol% Ga_2O_3 was sufficient to mount a potent bactericidal effect and demonstrated the potential of these glasses as a new therapeutic agent for pathogenic bacteria including the super bugs *MRSA* and *C. difficile*.

5. Melt Quenched Phosphate Based Glass Fibres

Glass fibres have potential applications in the engineering of soft tissues such as muscle and ligament due to a combination of suitable chemistry and morphology which can mimic the fibrous nature of these tissues [10]. It has been suggested that

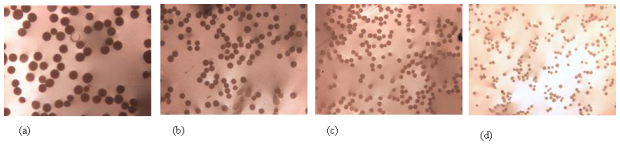


Figure 5: Phosphate glass fibres drawn with increasing drum speeds, which results in decreasing fibre diameter; (a) 35 ±5, (b) 25±3, (c) 16 ±2, and 11± 1 μm.

the glass fibres can act as a template with muscle cells growing along their long axis and forming myotubes; in particular, the three dimensional mesh arrangements have proven to be the best configuration for supporting cell attachment and proliferation [10, 64]. Glass fibre scaffolds with open mesh morphology allow for the diffusion of nutrient and waste products in and out of the construct, and permit the ingrowth of vasculatures and hence the tissue. They also provide the necessary structural support without compromising porosity [65-67]. Recently, it has been suggested that glass fibres could also act as a nerve conduit, since they can provide a guide for cell orientation, proliferation and growth [68, 69].

Phosphate glass fibres are conventionally fabricated by drawing from a high temperature melt. Typically fragments of the starting glass are remelted and fibres drawn from the melt onto a rotating collecting drum [66, 70]. Adjustment of the melt temperature is necessary to obtain a suitable viscosity for fibre drawing, since it is not feasible glasses with low melt viscosities to be drawn into fibres [10]. Additionally, the melt temperature should be above the glass crystallisation temperature; otherwise, fibre drawing becomes difficult [71] or the bioactivity of the glasses is reduced [72].

During the drawing process, the fibre diameter can easily be controlled by adjusting the drum speed: higher drum speed results in smaller fibre diameter as shown in Figure 5. From a biological standpoint, it has been reported that the fibre diameter has an effect on cell orientation [68]. It was found that as long as the fibre diameter is comparable to the size of the cell body, the cells will orientate along the long axis of fibre rather than around its circumference. Cells tend to wrap around the smaller diameter fibres, but in presence of less curvature, they can grow either perpendicular or parallel to the long axis. In such case, the fibres act as a contact guide, i.e. guide the cell growth; this is most useful for nerve regeneration since neuronal cells can be guided from both ends of the injured nerve in the right direction.

Fibre spacing (within a mess construct) can also be adjusted by changing the speed of the rotating drum. As previously mentioned, the inter-fibre spacing has an effect of the cell proliferation with the number of cells increasing as this spacing decreases [69]. A small fibre spacing makes it easy for cells to cross the gap between these fibres which reduces cell compaction of and prolongs proliferation. Moreover, a small inter-fibre spacing also increases the surface area for cells to attach and then proliferate.

One of the milestones in tissue engineering has been the development of 3D scaffolds that guide cells to form functional tissue. Tissue-engineered constructs that contain a controlled spatial distribution of cells and growth factors, as well as engineered scaffold materials with a well-defined microstructure, can now be fabricated [73]. Laying out phosphate glass fibres into two or three dimensional scaffolds has potential in this regard since such constructs have been shown to support cell attachment and proliferation [64].

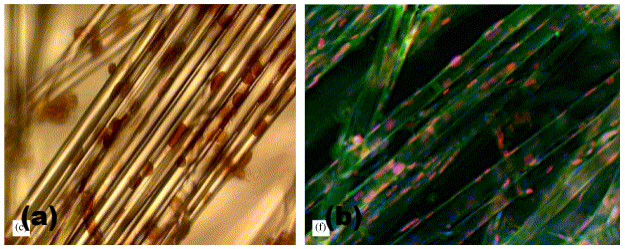


Figure 6: MPCs muscle cells (a) attached on iron phosphate glass fibres (BrdU staining was used to test the ability of attached MPCs to undergo replication.), and (b) fused and form multinucleate myotubes [Desmin, a cytoplasmic marker of all skeletal muscle cells, stained green, while Myogenin, a nuclear marker of differentiation, stained, and the nuclei stained blue] [10].

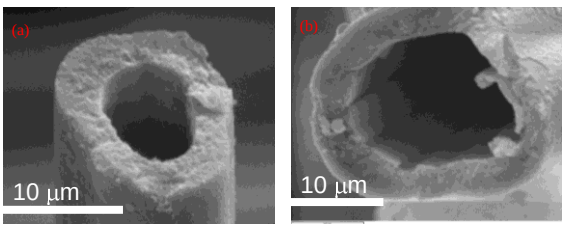


Figure 7: SEM images showing the tubular structures formed from glass fibres after 18 months of degradation; (a) 3 and (b) 5 mol% Fe_2O_3 containing glass fibres respectively [75].

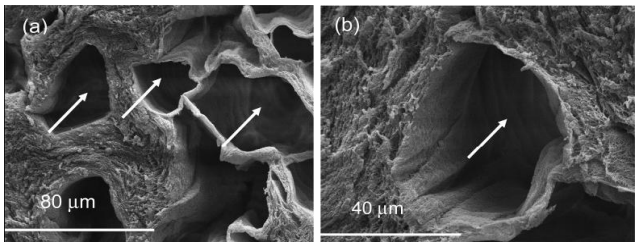
5.1. Fibres for Potential Soft Tissue Engineering Applications

A recent study of a 3D Phosphate glass fibre construct made from fibres with a composition of $(P_2O_5)62.9(Al_2O_3)21.9(ZnO)15.2$ demonstrated that it could support the proliferation and differentiation of human masseter muscle-derived cell cultures [64]. Parameters such as cell density, glass fibre configuration, growth factors and extracellular components were shown to be key factors in determining how well these glass fibres performed as an experimental scaffold material for engineered muscle tissue [64].

Phosphate glass fibres containing 5-22.5 wt % Fe_2O_3 have been used as reinforcing agents in the development of bioabsorbable composites designed for orthopedic applications. A cortical plug method was used to test the biocompatibility of these glasses; the results showed that no inflammation was observed over periods of up to five weeks [74].

Ahmed et al. [10] prepared and characterised iron containing glass fibres with different of composition $(P_2O_5)50(CaO)30(Na_2O)20\text{-}x(Fe_2O_3)x$ where x = 1-5. A dramatic improvement in immortal muscle precursor cell line attachment was observed for fibres with 4 and 5 mol % Fe_2O_3. The high cell density achieved is illustrated in Figure 6 [10].

Figure 8: SEM of a cross section through the phosphate



glass fibres-collagen scaffold showing (a) a cluster of channels left in the matrix as the fibres degrades, and (b) close up of a typical channel [76].

In another study, it was that fibres containing 3 mol % Fe_2O_3 are compatible with both primary human osteoblasts and fibroblasts, supporting a clear proliferation pattern, and permitting an even growth morphology [50].

Furthermore, Fe_2O_3 doped phosphate glass fibres have an intriguing ability of to form capillary-like channels as they degrade in aqueous media. Figure 7 shows images of these tubes viewed under an electron microscope. The degradation process which these glass fibres undergo is a combination of surface hydration and internal hydrolysis. Initially, the hydration of the outer surface of the fibres forms a protective barrier against degradation. Over longer periods, bulk degradation takes place by hydrolysis of the long Q^2 chains into shorter phosphate chains with Q^1 and Q^0 units dominating the structure of the resultant channels as evidenced from both FTIR and Raman spectroscopy [75].

Further to the previous findings, studies incorporating phosphate glass fibres containing 3 mol % Fe2O3 have also been used as templates for the in situ formation of uni-directionally aligned channels in 3D dense collagen scaffolds [76]. Assessment of diffusion through these scaffolds was made by recording the movement of micro-bubble agents through the construct using ultrasound and SEM imaging. The free movement of the coated micro-bubble agents confirmed that the channels were continuous in nature and 30-40 □ m in diameter (approximately the same fibre diameter) as shown in Figure 8. Moreover, this construct maintained excellent viability of human oral fibroblasts after 24 hours in culture, and the cells showed tightly packed spindle shaped appearance forming a three dimensional network; spreading over both the collagen matrix and the glass fibres with no preference for either of them.

Phosphate glass fibres with varying amounts of copper oxide, 0, 1, 5 and 10 mol% CuO, were produced for potential use in wound healing applications. The effect of two fibre diameters on short term (3 hours) attachment and killing against Staphylococcus epidermidis were investigated, and related to their rate of dissolution in deionised water and copper ion release. The results showed that there was a significant decrease in the rate of degradation both with increasing CuO content and increasing in fibre diameter. Over six hours, the amount of copper ions released increased with both increasing CuO content, and decreasing fibre diameter (i.e. increasing surface area to volume ratio). A decrease in the number of viable Staphylococci was observed both attached to the CuO containing fibres and in the surrounding environment [11].

6. Low Temperature Sol-gel Synthesis of Phosphate Based Glasses

6.1 Introduction to Sol-Gel Methods

The sol-gel process is a low temperature wet-chemical technique for the fabrication of oxide materials. The process starts with a chemical solution that reacts to produce colloidal particles; this solution is known as the sol. Typical precursors are inorganic alkoxides and metal chlorides, which undergo hydrolysis and polycondensation reactions to form a colloid. The sol evolves via further condensation reactions towards the formation of an inorganic network containing a liquid phase; this is known as the gel. Growth of an inorganic network occurs via the formation of M-O-M and M-OH-M bridges (where M is an electropositive element, typically Si, Ti, Al or Zr) which generates polymeric species throughout the solvent medium. A drying step serves to remove the liquid phase from the gel thus forming a porous material. Thermal treatment (often referred to as calcination) can be used to promote further polycondensation, leading to consolidation and densification of the material's structure. One of the main advantages of the sol-gel process is versatility: the precursor sol can be either deposited on a substrate to form a film (e.g. by dip-coating or spin-coating), cast into a suitable container with the desired shape (e.g. to obtain a monolithic ceramics, glasses, fibers, membranes, aerogels), or used to synthesize ultra-fine powders (e.g. microspheres, nanospheres) [77, 78].

The low temperature of the sol-gel process is generally below the crystallisation temperature of oxide materials which allows for the production or novel glasses and amorphous materials. This and the availability of suitable precursors has led to the publication of over 5000 papers concerning sol-gel derived silica based materials over the last 25 years. Many for these papers concern biomaterials and are a direct result of the discovery that silicate based bioactive glasses can be prepared by sol-gel methods [79]. The great potential of sol-gel chemistry in this regard is that the low temperature nature of the synthesis permits the inclusion of biologically active molecules that could not survive the high temperatures necessary in the preparation of glass biomaterials by melt quenching methods. To date, proteins [80, 81], antibiotics [82] and chemotherapy agents [83, 84] have all been successfully incorporated into silica based glass biomaterials using sol-gel chemistry: the porous nature of these materials allows controlled release of the biologically active molecules over long periods of time, thus offering the potential of sustained in-situ therapy. Furthermore, biocompatible polymers can also be included in the synthesis to produce materials with improved mechanical properties for use as tissue engineering scaffolds [85].

In contrast to the volume of work on silica based sol-gel biomaterials, there is much less on phosphate based biomaterials prepared by the same methods. Of the studies published so far, most concern the sol-gel preparation of powders or coatings of hydroxyapatite [86-89] or other crystalline calcium phosphates [90-92]. The examples focused on bioactive glasses are almost exclusively studies of phosphosilcates with a high silica content [93, 94].

6.2 Sol-Gel Synthesis of Phosphate Based Glasses

The reason for the dearth of literature examples of the sol-gel preparation of phosphate based glasses is that it is significantly more demanding than the preparation of silicate glasses by the same methods. The problem is finding the right phosphorus precursor: the hydrolysis of alkyl phosphates is very slow under sol-gel conditions and phosphate anions (e.g. PO_4^{3-}) tend to form precipitates rather than network structures based upon P–O–P bonding [95]. As a solution to this problem, Livage et al. [95] suggested using $PO(OH)_{3-x}(OR)_x$ (where R = alkyl group) precursors, obtained via the dissolution of P_2O_5 in alcohols. Using this method, they prepared some Ti-phosphates from clear sols with a P/Ti ratio of 1. Lee et al. also successfully reacted PO(OH)3-x(OR)x (R = ethyl or butyl, x = 1 or 2) with alkoxides of lithium, sodium, silicon, potassium and zinc to form a multicomponent glass [96]. The suitability of $PO(OH)_{3-x}(OR)_x$ compounds as sol-gel precursors was further illustrated by a study by Noda et al. [97]. This study focused on the effect of phosphorus sources on the synthesis of KTiOPO4 thin films by the sol-gel method. Triethylphosphate ($OP(OEt)_3$), phosphorus pentoxide (P_2O_5),di-n-butylphosphate ($(nBuO)_2PO(OH)$), ethylphosphonate ((EtO)P(OH)2), methylphosphonate ($(MeO)P(OH)_2$), and trimethylphosphonate ($(P(OMe)_3$) were all tested as starting phosphorus compounds. The results indicated that phosphorus compounds with hydroxy groups reacted with the Ti alkoxides to form Ti-O-P bonds, which prevented the undesirable evaporation of phosphorus compounds during heat treatment.

Some of the less favourable precursors phosphate have also successfully been employed in the synthesis of amorphous phosphate based materials. Makino et al. [98] prepared $Mg0.5Ti_2(PO4)_3$ gels from $Mg(CH_3COO)_2{\cdot}4H2O$ and $NH_4H_2PO_4$ in aqueous solution and $C_4H_9O[Ti(OC_4H_9)2O]_4C_4H_9$ in ethanol. Their samples remained amorphous even after heating to 500 oC. Tang et al. [99] prepared TiO_2-P_2O_5 glasses with potential photonics application from the reaction of triethylphosphate with titanium isopropoxide.

`Over the last five years, nearly all the work on sol-gel prepared phosphate based glasses has involved one of two synthetic routes, which have now emerged as almost the standard procedures. The first of these is the, previously described, reaction of a 1:1 molar mixture of $OP(OH)_2(OR)$ and $OP(OH)(OR)_2$ with reactive metal alkoxides and the second is the reaction of aluminium lactate with an aqueous phosphate solution. The former method has recently been used to prepare $(TiO_2)0.5(P_2O_5)0.5$ gels and glasses which have potential applications in humidity sensors and photonics, respectively [100]. The latter method has been successfully used to prepare clear, monolithic Al_2O_3-P_2O_5 and Na_2O-Al_2O_3-P_2O_5 gels by reacting aluminium lactate with phosphoric acid solution or sodium polyphosphate solution, respectively [101, 102]. Such glasses are useful in a number of applications including as catalysts and catalyst supports, laser devices, solid-state batteries, and hermetic seals to materials with high thermal expansion coefficients. This sol-gel synthesis has recently been used to extend the glass forming region of the B_2O_3-Al_2O_3-P_2O_5 system [103] and to prepare aluminium fluoride phosphate glasses [104].

6.3 Phosphate Based Sol-Gel Biomaterials

The first sol-gel phosphate based glasses specifically aimed at biomedical applications were synthesized by Carta et al. [105]. CaO-Na_2O-P_2O_5 glasses were prepared by reacting mono- and di-substituted ethylphosphate with alkoxides of the calcium and sodium in ethylene glycol. This method, however, has significant disadvantages in that relatively high temperatures are required to remove the ethylene glycol solvent from the gels and the resultant glasses do not exhibit significant porosity, possibly as a result of the necessary heat treatment. Recently, a new sol-gel route to phosphate-based materials that produces glassy gels at lower temperatures than previously reported has been developed [106]. Samples were prepared by the reaction of a 1:1 molar mixture of mono- and di-substituted n-butylphosphate with sodium methoxide and calcium methoxyethoxide in an alcohol solvent mix. Sructural characterisation of the samples was carried out using a combination of thermal analysis, FTIR, 31P solid state NMR and high-energy XRD. The results demonstrated that hydrated $(CaO)0.3(Na_2O)0.2(P_2O_5)0.5$ samples with structures comparable to their melt-quenched counterparts could be prepared with a maximum processing temperature in the range 200-250 °C. The main structural difference between the melt-quenched and the sol-gel samples was that the latter were partially hydrated. The results also suggest that the reactive nature of the sodium methoxide and calcium methoxyethoxide helps promote P-O-P linkages during the sol-gel reation. Furthermore, it was shown that this method can be used to produce porous foams, which have potential applications as tissue engineering scaffolds.

In a related study, CaO-TiO_2-P_2O_5 glasses for potential biomedical applications were prepared by a similar sol-gel method [107]. The structure of samples were characterised using high enrgy X-ray diffraction [107] and Ti K-edge XANES [108].

The recent developments described above encourage further exploitation of sol-gel chemistry in the preparation of phosphate based biomaterials. In particular, it should now be possible to include biologically active molecules that are not stable to high temperature, such as proteins, antibiotic and other drugs, in the synthesis. Bioactive polymers could also be included to improve the mechanical properties of the resultant materials, thus providing materials with improved properties for use in tissue engineering constructs. Finally, there exists the potential to coat biomedical devices with a sol-gel derived antimicrobial coating via the inclusion of biocidal metal ions such as Ag+ and Ga3+.

7. Outlook for Phosphate Based Glasses

Zinc phosphate glasses could be potentially be used for the treatment of chronic inflammatory diseases such as Crohn's disease and rheumatoid arthritis, which are both characterised by decreased Zn^{2+} levels in the blood.

Phosphate glass fibres could be used as a vehicle for cell delivery to inaccessible areas – e.g. for the delivery of

periodontal ligament cells in the treatment of advanced periodontitis.

Phosphate based glass fibres with antimicrobial properties could be prepared in a mesh form for use as a wound dressing for the treatment of sever burns, leg ulcers, pressure sores, and infected surgical wounds, providing both protection against the ingress of micro-organisms and releasing antimicrobial ions (e.g. Cu^{2+}, Ag^{+} and Ga^{3+}) as they dissolve to help combat infection. Such meshes would be used on a temporary bases with the highly degradable nature of the fibres is benefiting the release of antimicrobial agents. These fibrescould also be incorporated into bone cements used in the fixation of orthopaedic devices such as replacement hips. The antibacterial ions released from the bone cement into the tissue surrounding the replacement device could help control the number of bacteria left in the operative wound.

The ability of phosphate glass fibres to form microtubes as they degrade formation through the degradation of these could potentially be applied to a number of areas including drug delivery and cell transportation, e.g. to act as a conduit during nerve healing by transporting nerve cells. Moreover, they could be used in combination with either natural or synthetic polymers to help the in-growth of vascularisation and the diffusion of nutrient and waste through 3D scaffolds for soft and hard tissue engineering: e.g., the engineering of muscle, ligament, tendon, or bone. It would also be possible to fabricate one construct containing fibres with different degradation rates so that the rapidly degrading fibres could provide *in situ* channels for the rapid growth of blood vessels, and the more stable fibres could aid the alignment of cells to form the proposed tissue.

Recent developments in phosphate based sol-gel chemistry, now mean that biologically active molecules that are not stable to high temperature, such as proteins, antibiotic and other drugs, can be included in the synthesis. The resultant materials have potential to be used in devices to target the delivery of such molecules in the human body and provide controlled, sustained *in-situ* therapy. Bioactive polymers could also be included in the synthesis to produce materials with improved mechanical properties for use in tissue engineering constructs. Finally, there exists the potential to coat biomedical devices with a sol-gel derived antimicrobial coating via the inclusion of biocidal metal ions such as Ag^{+} and Ga^{3+}.

Acknowledgments

The authors would like to acknowledge the financial support from the EPSRC.

Notes and references

[a]*Division of Biomaterials and Tissue Engineering, UCL Eastman Dental Institute, 256 Gray's Inn Road, London, WC1X 8LD. Fax +44 (0) 207 915 1227. Tel: +44(0) 207 915 1189.*
[+] *Email:j.knowles@eastman.ucl.ac.uk.*
**Email:e.abouneel@eastman.ucl.ac.uk..*
bSchool of Physical Sciences, University of Kent, Canterbury, Kent, CT2 7NH. Fax: +44 (0)1227 827558. Tel: +44 (0)1227 827887.
Email: r.j.newport@kent.ac.uk

1. LL. Hench, JM. Polak. Science 2002, 295 (5557), 1014.
2. LL. Hench, JK. West. Life Chem Rep., 1996, 13,187.
3. P. Saravanapavan, JR. Jones, RS. Pryce, LL Hench. J Biomed Mater Res., 2003, 66, 110.
4. JE Gough, I Nottingher, LL Hench. J Biomed Mater Res., 2004, 66A, 640.
5. RM. Day, AR. Boccaccini. J Biomed Mater Res., 2005, 73A (1), 73.
6. V. Salih, K. Franks, M. James, GW. Hastings, JC. Knowles. Journal of Material Science: Materials in Medicine, 2000, 11, 615.
7. ES. Tadjoedin, GL. de Lange, DM. Lyaruu, L. Kuiper, EH. Burger. Clin. Oral Implants Res., 2002, 13 (4), 428.
8. ES. Tadjoedin, GL. de Lange, PJ. Holzmann, L. Kulper, EH. Burger. Clin. Oral Implants Res., 2000, 11(4),334.
9. J C. Knowles. J Mater Chem., 2003, 13, 2395.
10. I. Ahmed, CA. Collins, M. Lewis, I. Olsen, JC. Knowles. Biomaterials, 2004, 25, 3223.
11. EA. Abou Neel, I. Ahmed, J. Pratten, SN. Nazhat, JC. Knowles. Biomaterials, 2005, 26, 2247.
12. EA. Abou Neel, I. Ahmed, JJ. Blaker, A. Bismarck, AR. Boccaccini, MP. Lewis, SN. Nazhat, JC. Knowles. Acta Biomaterialia, 2005, 1, 553.
13. U. Hoppe. Journal of Non-Crystalline Solids, 1996, 195, 138.
14. WH. Zachariasen. American Chemical Society, 1932, 54(3), 3841.
15. GN Greaves, W. Smith, E. Giulotto, E. Pantos. Journal of Non-Crystalline Solids, 1997, 222, 13.
16. BS. Bae, MC. Weinberg. J. Am. Ceram. Soc., 1991, 74(12), 3039.
17. BC. Sales, LA. Boatner, JO. Ramey. Journal of Non-Crystalline Solids, 2000, 263&264, 155.
18. D C. Clupper, JE. Gough, PM. Embanga, I. Notingher, LL. Hench. Journal of Material Science: Material in Medicine, 2004, 15, 803.
19. ME. Milberg, MC. Daly. The Journal of Chemical Physics. 1963, 39(11), 2966.
20. JB Murgatroyd. Journal of the Society of Glass Technology, 1948, 32, 291.
21. F. Delahaye, L. Montagne, G. Palavit, JC. Touray, P. Baillif, Journal of Non-Crystalline Solids 1998, 242, 25.
22. RJ. Kirkpatrick, RK. Brow, Solid State Nuclear Magnetic Resonance, 1995, 5, 9.
23. RK. Brow. Journal of Non-Crystalline Solids, 2000, 263 & 264, 1.
24. G. Walter, J. Vogel, U. Hoppe, P. Hartmann. Journal of Non-Crystalline Solids, 2001, 296, 212.
25. G. Walter, U. Hoppe, T. Baade, R. Kranold, D. Stachel. Journal of Non-Crystalline Solids, 1997, 217, 299.
26. U. Hoppe, G. Walter, R. Kranold, D. Stachel. Journal of Non-Crystalline Solids, 2000, 263&264, 29.
27. JR. Van Wazer., Interscience, New York, vol 1,1985.
28. J. Schneider, SL. Oliveira, LAO. Nunes, F. Bonk, H. Panepucci. Inorg Chem., 2005, 44; 423.
29. GK. Marasinghe, M. Karabulut, CS. Ray, DE. Day, DK. Shuh, PG. Allen, ML. Saboungi, M. Grimsditch, D. Haeffner. Journal of Non-Crystalline Solids, 2000, 263&264, 146.
30. MG. Mesko, DE. Day. Journal of Nuclear Materials, 1999, 273; 27.
31. X. Fang, CS. Ray, GK. Marasinghe, DE. Day. Journal of Non-Crystalline Solids 2000, 263&264, 292.
32. CA. Ray, X. Fang, GK. Karabulut, DE. Day. Journal of Non-Crystalline Solids, 1999, 249, 1.
33. H. Gao, T. Tan, D. Wang, Journal of Controlled Release 2004, 96, 21.
34. T. Gilchrist, DM. Healy, C. Drake. Biomaterials 1991, 12, 76.
35. CF. Drake. Consultation on Immunomodulation. 1985, Bellagio, Italy, 16-18 April.
36. C. Curless, J. Baclaski, R. Sachdev. Biotechnol Prog, 1996, 12(1), 22.
37. SB. Telfer, DV. Illingworth, PJB. Anderson, G. Zervas, G. Carlos. Biochemical Society Transactions1985, 13, 529.
38. WM. Allen, CF. Drake, M. Tripp. TEMA 1984, 5, 29th June-4th July, Aberdeen.
39. WM. Allen, BF. Sansom, CF. Drake, PR. Moore. In Y. Ruchebusch, P-L. Toutain, GD. Korltz. Proceedings from the 2nd European Association for Veterinary Pharmacology and Toxicology, 1983, Toulouse, 13-17th September, MTP Press Limited , Boston, p.183.
40. TN. O'sullivan, JD. Smith, JD. Thomas, C. Drake. Environ. Sci. Technol 1991, 25, 1088.

41. SH. Cartmell, PJ. Dorthy, JA. Hunt, DM. Healy, T. Gilchrist Journal of Material Science: Material in Medicine 1998, 9, 773.
42. K Franks. PhD thesis, University of London, 2000.
43. AJ. Parsons, LD. Burling, CA. Scotchford, GS. Walker, CD. Rudd. Journal of Non-Crystalline Solids 2006, (352) 50-51, 5309.
44. EA. Abou Neel, I. Ahmed, J. Pratten, SN. Nazhat, JC. Knowles. Biomaterials 2005, 26, 2247.
45. T. Fujita, N. Izumo, R. Fukuyama, T. Meguro, H. Nakamuta, T. Kohno, M. Koida. Biochem Biophysic Res Commun, 2001, 280, 348.
46. JE Gough, P. Christian, CA. Scotchford, CD. Rudd, IA. Jones. J Biomed Mater Res, 2002, 59(3), 481.
47. JE. Gough, P. Christian, CA. Scotchford, IA. Jones. J Biomed Mater Res, 2003, 66A, 233.
48. M. Uo, M. Mizuno, Y. Kuboki, A. Makishima, F. Watari. Biomaterials 1998, 19, 2277.
49. K. Franks, I. Abrahams, JC. Knowles. Journal of Material Science: Materials in Medicine 2000, 11, 609.
50. M. Bitar, V. Salih, V. Mudera, JC. Knowles, M. Lewis. Biomaterials 2004, 25, 2283.
51. JC. Knowles, K. Franks, I. Abrahams. Biomaterials 2001, 22, 3091.
52. K. Franks, V. Salih, JC. Knowles. Journal of Material Science: Materials in Medicine 2002, 13, 549.
53. V. Salih, A. Patel, JC. Knowles. Biomed Mater, 2007, 2, 1.
54. EA. Abou Neel, T. Mizoguchi, M. Ito, M. Bitar, V. Salih, JC. Knowles. Biomaterials 2007, 28, 2967.
55. EA. Abou Neel, JC. Knowles. J Materials Science: Materials in Medicine 2007, DOI 10.1007/s10856-007-3079-5.
56. EA. Abou Neel, LA. O'Dell, W. Chrzanowski, ME. Smith, JC. Knowles. Submitted to Biomedical Materials Research: Part B.
57. TJ. Webster, C. Ergun, RH. Doremus, RW. Siegel, R. Bizios. J Biomed Mater Res, 2000, 51, 475.
58. AG. Gristina. Science 1987, 237, 588.
59. AM. Mulligan, M. Wilson, JC. Knowles. Biomaterials 2003a, 24(10), 1797.
60. AM. Mulligan, M. Wilson, JC. Knowles. J Biomed Mater Res, 2003b, 67A, 401.
61. I. Ahmed, D. Ready, M. Wilson, JC. Knowles. J Biomed Mater Res, 2006, 78A, 618.
62. SP. Valappil, DM. Pickup, DL. Carroll, CK. Hope, J. Pratten, RJ. Newport, ME. Smith, M. Wilson, JC. Knowles. Antimicrob. Agents Chemother 2007, 51, 4453.
63. SP. Valappil, D. Ready, EA. Abou Neel, DM. Pickup, W. Chrzanowski, LA. O'Dell , RJ. Newport, ME. Smith, M. Wilson, JC. Knowles. Adv Fun Mater 2008, 18, 732.
64. R. Shah, ACM. Sinanan, JC. Knowles, NP. Hunt, MP. Lewis. Biomaterials 2005, 26, 1497.
65. DJ. Mooney, CL. Mazzoni, C. Breuer, K. McNamara, D. Hern, JP. Vacanti, R. Langer. Biomaterials 1996, 17, 115.
66. MA. De Diego, NJ. Coleman, LL. Hench. J Biomed Mater Res, 2000, 53, 199.
67. RZ Domingues, AE. Clark, AB. Brennan. J Biomed Mater Res, 2001, 55, 468.
68. SJ. Lee, JH. Lee, HB. Lee. Korea Polymer Journal 1999a, 7(2), 102.
69. BM. Hatcher, CA. Seegert, AB. Brennan. J Biomed Mater Res, 2003, 66A, 840.
70. J. Choueka, JL. Charvert, H. Alexander, YH. Oh, G. Joseph, NC. Blumenthal, WC. LaCourse. J Biomed Mater Res, 1995, 29, 1309.
71. DC. Clupper, JE. Gough, MM. Hall, AG. Clare, WC. La Course, LL. Hench. J Biomed Mater Res, 2003, 67A, 285.
72. RL. Orifice, LL. Hench, AE. Clark, AB. Brennan. J Biomed Mater Res, 2001, 55, 460.
73. DM. Hutmacher1, M. Sittinger, MV. Risbud. TRENDS in Biotechnology 2004; 22 (7): 354.
74. ST. Lin, SL. Krebs, S. Kadiyala, KW. Leong, WC. LaCourse, B. Kumar. Biomaterials 1994,15(13), 1057.
75. EA. Abou Neel, AM. Young, SN. Nazhat, JC. Knowles. Advanced Materials. 2007, 19; 2856.
76. SN. Nazhat, EA. Abou Neel, A. Kidane, I. Ahmed, C. Hope, M. Kershaw, PD. Lee, E. Stride, N. Saffari, JC. Knowles, RA. Brown. Biomacromolecules 2007, 8, 543.
77. CJ. Brinker, GW. Scherer. The Physics and Chemistry of Sol–Gel Processing. Academic Press, New York, 1990.
78. LL. Hench, JK. West. Chemical Reviews 1990, 90, 33.
79. R. Li, AE. Clark, LL. Hench. J. Appl. Biomater. 1991, 2(4), 231.
80. EM. Santos, S. Radin, P. Ducheyne. Biomaterials 1999, 20(18), 1695.
81. RB. Bhatia, CJ. Brinker, AK. Gupta, AK. Singh. Chem. Mater. 2000, 12(8), 2434.
82. S. Radin, P. Ducheyne, T. Kamplain, BH. Tan. J. Biomed. Mater. Res. 2001, 57(2):313.
83. S. Fireman-Shoresh, N. Husing, D. Avnir. Langmuir 2001, 17(19), 5958.
84. K. Czarnobaj, J. Lukasiak. Drug Delivery, 2004, 11(6), 341.
85. MM. Pereira, JR. Jones, LL. Hench. Advances in Applied Ceramics 2005, 104(1), 35.
86. B. Ben-Nissan, A. Milev, R. Vago. Biomaterials 2004, 25(20), 4971.
87. CS. Chai, KA. Gross, B. Ben-Nissan. Biomaterials 1998, 19(24), 2291.
88. HW. Kim, YM. Kong, CJ. Bae, YJ. Noh, HE. Kim. Biomaterials 2004, 25(15), 2919.
89. CM. Lopatin, V. Pizziconi, TL. Alford, T. Laursen. Thin Solid Films 1998, 326(1-2), 227.
90. SJ. Kalita, A. Bhardwaj, HA. Bhatt. Materials Science & Engineering C-Biomimetic and Supramolecular Systems 2007, 27(3), 441.
91. L. Gan, R. Pilliar. Biomaterials 2004, 25(22), 5303.
92. L. Gan, H. Wang, A. Tache, N. Valiquette, D. Deporter, R. Pilliar. Biomaterials 2004, 25(22), 5313.
93. S. Padilla, J. Roman, A. Carenas, M. Vallet-Regi. Biomaterials 2005, 26(5), 475.
94. A. Balamurugan, G. Ballossier, J. Michel, S. Kannan, H. Benhayoune, AHS. Rebelo, et al. J. Biomed. Mater. Res. B Appl. Biomater. 2007, 83B, 546.
95. J. Livage, P. Barboux, MT. Vandenborre, C. Schmutz, F. Taulelle. J. Non-Cryst Solids 1992, 147, 18.
96. BI. Lee, WD. Samuels, LQ. Wang, GJ. Exarhos. J. Mater. Res. 1996, 11, 134.
97. K. Noda, W. Sakamoto, K. Kikuta, T. Yogo, S. Hirano. Chem. Mater. 1997, 9(10), 2174.
98. K. Makino, Y. Katayama, T. Miura, T. Kishi. J. Power Sources 2001, 99(1-2), 66.
99. AJ. Tang, T. Hashimoto, T. Nishida, H. Nasu, K. Kamiya. J. Ceram. Soc. Jpn. 2004, 112(1309), 496.
100. DM. Pickup, RJ. Speight, JC. Knowles, ME. Smith, RJ. Newport. Mater. Res. Bull. 2008, 43, 333.
101. Zhang L, Eckert H (2004), 'Sol-gel synthesis of Al2O3-P2O5 glasses: mechanistic studies by solution and solid state NMR', J. Mater. Chem. 14(10), 1605-1615.
102. Zhang L, Chan J C C, Eckert H, Helsch G, Hoyer L P, Frischat G H (2003), 'Novel sol-gel synthesis of sodium aluminophosphate glass based on aluminum lactate', Chem. Mater. 15(14), 2702-2710.
103. L. Zhang, H. Eckert. J. Mater. Chem. 2005, 15(16), 1640.
104. L. Zhang, CC. de Araujo, H. Eckert. Chem. Mater., 2005, 17(12), 3101.
105. D. Carta, DM. Pickup, JC. Knowles, ME. Smith, RJ. Newport. Journal of Materials Chemistry 2005, 15, 2134.
106. DM. Pickup, P. Guerry, RM. Moss, JC. Knowles, ME. Smith, RJ . Newport. J. Mater. Chem. 2007, 17, 4777.
107. DM. Pickup, KM. Wetherall, JC. Knowles, ME. Smith, RJ. Newport Journal of Material Science: Materials in Medicine 2008, 19, 1661.
108. DM. Pickup, EA. Abou Neel, RM. Moss, KM. Wetherall, P. Guerry, ME. Smith, JC. Knowles, RJ. Newport. Journal of Material Science: Materials in Medicine, 2008, 19, 1681.