Polymer/bioactive glass nanocomposites for biomedical applications. A Review

Aldo R. Boccaccini, Melek Erol, Wendelin J. Stark, Dirk Mohn, Zhongkui Hong, João F. Mano

► To cite this version:

Aldo R. Boccaccini, Melek Erol, Wendelin J. Stark, Dirk Mohn, Zhongkui Hong, et al.. Polymer/bioactive glass nanocomposites for biomedical applications. A Review. Composites Science and Technology, 2010, 70 (13), pp.1764. 10.1016/j.compscitech.2010.06.002 . hal-00681659

HAL Id: hal-00681659

<https://hal.science/hal-00681659>

Submitted on 22 Mar 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Polymer/bioactive glass nanocomposites for biomedical applications. A Review

Aldo R. Boccaccini, Melek Erol, Wendelin J. Stark, Dirk Mohn, Zhongkui Hong, João F. Mano

PII: S0266-3538(10)00228-9
DOI: [10.1016/j.compscitech.2010.06.002](https://doi.org/10.1016/j.compscitech.2010.06.002)
Reference: CSTE 4737

To appear in: *Composites Science and Technology*

Received Date: 29 December 2009
Revised Date: 30 May 2010
Accepted Date: 2 June 2010

Please cite this article as: Boccaccini, A.R., Erol, M., Stark, W.J., Mohn, D., Hong, Z., Mano, J.F., Polymer/bioactive glass nanocomposites for biomedical applications. A Review, *Composites Science and Technology* (2010), doi: [10.1016/j.compscitech.2010.06.002](https://doi.org/10.1016/j.compscitech.2010.06.002)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Polymer/bioactive glass nanocomposites for biomedical applications. A Review

Aldo R. Boccaccini^{1,2*}, Melek Erol¹, Wendelin J. Stark³, Dirk Mohn³, Zhongkui Hong⁴,
João F. Mano^{5,6*}

¹Department of Materials, Imperial College London
Prince Consort Road, London SW7 2BP, United Kingdom

²Institute of Biomaterials, Department of Materials Science and Engineering, University
of Erlangen-Nuremberg,
Cauerstr. 6, 91058 Erlangen, Germany

³Institute for Chemical and Bioengineering, ETH Zurich,
Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland

⁴Physics and Astronomy Department,
University of Missouri-Columbia, Columbia, MO 65211, USA

⁵3Bs Research Group-Biomaterials, Biodegradables and Biomimetics, AvePark, Zona
Industrial da Gandra, S. Cláudio do Barco, 4806-909 Caldas das Taipas – Guimarães,
Portugal

⁶IBB – Institute for Biotechnology and Bioengineering, PT Government Associated
Laboratory, Guimarães, Portugal

* Corresponding authors:

aldo.boccaccini@ww.uni-erlangen.de
jmano@dep.uminho.pt

Abstract

Nanoscale bioactive glasses have been gaining attention due to their superior osteoconductivity when compared to conventional (micron-sized) bioactive glass materials. The combination of bioactive glass nanoparticles or nanofibers with polymeric systems enables the production of nanocomposites with potential to be used in a series of orthopedic applications, including tissue engineering and regenerative

medicine. This review presents the state of art of the preparation of nanoscale bioactive glasses and corresponding composites with biocompatible polymers. The recent developments in the preparation methods of nano-sized bioactive glasses are reviewed, covering sol-gel routes, microemulsion techniques, gas phase synthesis method (flame spray synthesis), laser spinning, and electro-spinning. Then, examples of the preparation and properties of nanocomposites based on such inorganic bionanomaterials are presented, obtained using various polymer matrices, including polyesters such as poly(hydroxybutyrate), poly(lactic acid) and poly(caprolactone), and natural-based polymers such as polysaccharides (starch, chitin, chitosan) or proteins (silk, collagen). The physico-chemical, mechanical, and biological advantages of incorporating nanoscale bioactive glasses in such biodegradable nanocomposites are discussed and the possibilities to expand the use of these materials in other nanotechnology concepts aimed to be used in different biomedical applications are also highlighted.

KEYWORDS: A. Nanoparticles; A. Bioactive glass; A. Nanocomposites; A. Particle-reinforced composites; B. Porosity/Voids

1. Introduction

Bioactive glasses of silicate composition, which were first developed by Hench and co-workers in 1969 [1], represent a group of surface reactive materials which are able to bond to bone in physiological environment [2]. Bioactive glasses most widely used in biomedical applications consist of a silicate network incorporating sodium, calcium and phosphorus in different relative proportions. The classical 45S5 bioactive glass composition universally known as Bioglass® (composition in wt%: 45% SiO₂, 24.5% Na₂O, 24.5% CaO and 6% P₂O₅), for example, has approval of the US Food and Drug Administration (FDA) and is used in clinical treatments of periodontal diseases as bone filler as well as in middle ear surgery [2]. Other bioactive glass compositions contain no sodium or have additional elements incorporated in the silicate network such as fluorine, magnesium, strontium, iron, silver, boron, potassium or zinc [3-9].

Fabrication techniques for bioactive glasses include both traditional melting methods and sol-gel techniques [2-4, 10]. The typical feature common to all bioactive glasses, being melt or sol-gel derived, is the ability to interact with living tissue forming strong bonds to bone (and in some cases soft) tissue, a property commonly termed bioreactivity or bioactivity [2]. The bonding to bone is established by the precipitation of a calcium-deficient, carbonated apatite surface layer on the bioactive glass surface when in contact with relevant physiological fluid or during *in vivo* applications. It is now widely accepted that for establishing bond with bone, such biologically active apatite surface layer must form at the material/bone interface [2, 11]. The development of these bioactive apatite layers is the common characteristic of all known inorganic materials used for orthopedic implants, bone replacement and bone tissue engineering scaffolds [2, 12]

Early applications of bioactive glasses were in the form of solid pieces for small bone replacement, i.e. in middle ear surgery [2]. Later, other clinical applications of bioactive glasses were proposed, for example in periodontology [13, 14], endodontology [15, 16] or as coating on metallic orthopedic implants [17, 18]. More recently, great potential has been attributed to the application of bioactive glasses in tissue engineering and regenerative medicine [12, 19-22]. Bone tissue engineering is one of the possible most exciting future clinical applications of bioactive glasses, e.g. to fabricate optimal scaffolds with osteogenic and angiogenic potential [22]. Both micron-sized and nanoscale particles are considered in this application field, which includes also the fabrication of composite materials, e.g. combination of biodegradable polymers and bioactive glass [12, 20, 23], as discussed in detail further below. Moreover the surface modification of such biodegradable composites with smart polymers allows to produce substrates in which biomineralization could be triggered by the action of external stimuli, such as temperature or pH [24, 25]. In this context, bioactive silicate glasses exhibit several advantages in comparison to other bioactive ceramics, e.g. sintered hydroxyapatite. For example, it has been demonstrated that dissolution products from bioactive glasses upregulate the expression of genes that control osteogenesis [19], which explains the higher rate of bone formation in comparison to other inorganic ceramics such as hydroxyapatite [26]. Further studies using 45S5 Bioglass® particles have shown encouraging results regarding potential angiogenic

effects of Bioglass®, i.e. increased secretion of vascular endothelial growth factor (VEGF) in vitro and enhancement of vascularisation in vivo [27-29]. In addition, the incorporation of specific ions in the silicate network, such as Ag and Zn, has been investigated in order to develop antibacterial materials [30-32]. Bioactive glasses can also serve as vehicle for the local delivery of selected ions which can act to control specific cell functions, for example Co addition to suppress cell hypoxia [33]. Bioactive glasses are also being considered as haemostatic agents. For example, Bioglass® has been shown to reduce the clotting time of blood by 25% in laboratory tests (Lee-White Coagulation), with ionic release of calcium (Clotting factor IV) being considered a reason for its haemostatic properties. [34]. Moreover ferromagnetic bioactive glasses and glass-ceramics containing magnetite are being developed for hyperthermia treatment of cancer [35].

The range of bioactive glasses exhibiting these attractive properties has been extended over the years, in terms of both chemical composition and morphology, as new preparation methods have become available. In addition, all the specific effects and advantages of bioactive glasses mentioned above, including surface bioreactivity, can be enhanced or modified and controlled to a greater extent, if nanoparticles (or nanofibres) are available, as opposed to conventional micron-sized powders. This is relevant both for bioactive glasses used in particulate form as coatings in biomedical devices or as filler in composite materials, e.g. as biodegradable implants, dental fillers, tissue engineering scaffolds, tissue guidance membranes or drug delivery systems.

Bioactive glass/biodegradable polymer composite materials have emerged recently as new family of bioactive materials with applications ranging from structural implants to tissue engineering scaffolds [12]. These composites exploit the flexibility of polymers with the stiffness, strength and bioactive character of the bioactive glass fillers. So far, most work on this class of composites has been carried out using conventional (micron-size) bioactive glass particles as fillers (or coatings) [12]. However, recent research to be reviewed in this paper demonstrates the application of nano-sized bioactive glass particles and nanofibres (which have become available only in the last few years), in a range of novel composites with improved performance for biomedical applications, in particular tissue engineering and regenerative medicine.

Thus the topic of biodegradable/bioactive glass nanocomposites will be the subject of this review, which covers the available literature on production and characterization of nano-structured bioactive silicate glasses and their application in nanocomposites for biomedical applications. Section 2 discusses the key characteristics of nanoscale bioactive glasses. Different synthesis methods for bioactive glass nanoparticles and nanofibres are reviewed in Section 3. In Section 4, a comprehensive review of composite systems incorporating bioactive glass nanoparticles or nanofibres is presented while in Section 5 the state of the art is summarized and the scope for further research developments in the field is highlighted.

2. Characteristics of nanoscale bioactive glasses

A reduction in size to the nanometer scale of bioactive glass particles (or fibres) leads to a new family of nanostructured biomaterials which, combined with polymer matrices to form composites, are expected to exhibit enhanced performance in existing biomedical applications, leading also to new application opportunities. The higher specific surface area of nanoscale bioactive glasses allows not only for a faster release of ions but also a higher protein adsorption and thus enhanced bioactivity can be expected. There is evidence in the literature that faster deposition or mineralization of tissues such as bone or teeth is possible when these tissues are in contact with nanoscale particles, as opposed to micron-sized particles, considering that the bone structure exhibits nanoscale features consisting of a tailored mixture of collagen fibrils and hydroxyapatite nanocrystals [36, 37]. Mimicking the nanofeatures of bone on the surface of a synthetic implant material, for example, has been shown to increase bone-forming cell adhesion and proliferation [37]. These results have been obtained on TiO₂ and hydroxyapatite but the findings should be directly applicable to bioactive glasses too.

For bone tissue engineering purposes, where polymer/bioactive glass composite scaffolds are of great interest [12, 20, 38], the use of nanoscale bioactive glasses is expected to improve both mechanical and biological properties of scaffolds. Not only the surface bioreactivity of nanoparticles is higher than that of μm -size particles but also bioactive glass nanoparticles will induce nanostructured features on scaffold surfaces,

which are likely to improve osteoblast cell attachment and subsequent cell behavior. Other advantages of the reduced size of the inorganic particles include the possibility to use them to reinforce polymeric nanofibers, to process thin bioactive coatings or in injectable systems [39].

3. Fabrication techniques for bioactive glass nanoparticles and nanofibres

In the last few years silicate bioactive glass nanoparticles and nanofibres have become available and they are starting to be used in a range of biomedical applications in combination with polymers, forming nanocomposites. The success of the work carried out so far and the potential applications of these novel materials have prompted the preparation of the present review. In this Section the processing methods to fabricate nanoscale bioactive glasses are discussed.

3.1. Sol-gel techniques

The sol-gel process has a long history of use for synthesis of silicate systems and other oxides and it has become a widely spread research field with high technological relevance, for example for the fabrication of thin films, coatings, nanoparticles and fibres [10, 40, 41, 42]. As a typical liquid phase synthesis method, sol-gel usually involves the use of metal-organic precursors which are converted to inorganic materials either in water or in an organic solvent. The sol-gel synthesis of pure silica glass nanoparticles is well known [40]. However silica nanoparticles are not considered to belong to the family of bioactive glasses [2] and they will not be discussed in detail in this review. Applications in the biomedical field for silica nanoparticles have been discussed in the literature (see for example refs. [43-46]).

The synthesis of specific silicate bioactive glasses by the sol-gel technique at low temperatures using metal alkoxides as precursors was shown in 1991 by Li et al. [10]. For the synthesis of bioactive glasses, typical precursors used are tetraethyl orthosilicate, calcium nitrate and triethylphosphate. After hydrolysis and polycondensation reactions a gel is formed which subsequently is calcined at 600-700°C to form the glass. Based on the preparation method, sol-gel derived products, e.g. thin films or particles, are highly porous exhibiting a high specific surface area [10, 40, 46].

Recent work on fabricating bioactive silicate glass nanoparticles by sol-gel process has been carried out by Hong et al. [47]. In their research, nanoscale bioactive glass particles were obtained by the combination of two steps; sol-gel route and coprecipitation method, wherein the mixture of precursors was hydrolyzed in acidic environment and condensed in alkaline condition separately, and then followed by a freeze-drying process. A schematic diagram about the improved sol-gel synthesis process developed by Hong et al. [42, 47] is presented in Figure 1. The morphology and size of bioactive glass nanoparticles could be tailored by varying the production conditions and the feeding ratio of reagents [48, 49]. Figure 2 shows SEM micrographs of the produced nanoparticles with different shape and formulations.

It is important to control the surface morphology of the bioactive glass nanoparticles (NBG) in order to obtain the desired biological properties. Chen et al. [50] investigated the effects of different morphologies on the *in vitro* bioactivity of nano-sized bioactive glass particles in the system $\text{CaO-P}_2\text{O}_5\text{-SiO}_2$ by using lactic acid (LA) in the sol-gel process. It was reported that the addition of lactic acid decreased the particles size of the bioactive glass nanoparticles as seen from TEM micrographs (Figure 3). The surface morphologies with narrow unimodal or bimodal pore distribution resulted in a significant improvement in the *in vitro* bioactivity of NBGs compared to that of a smooth surface. It was concluded that not only the specific surface area and the pore size but also the surface morphology play an important role in influencing the *in vitro* bioactivity of NBGs [50]. Chen et al.[51] have also developed surface modified bioactive glass nanoparticles to improve their dispersibility by using a wet mechanical grinding technique. The particle size distribution of the sol-gel derived bioactive glass nanoparticles modified by a silane coupling agent was between 20 and 70 nm.

According to the FTIR and XPS results, silane chain covalently grafted onto the surface of nanoparticles. It was also reported that the layer of silane prevented the conglomeration behavior of sol-gel derived bioactive glass nanoparticles [51].

As mentioned above, different ions have been added to bioactive glasses, such as zinc, magnesium, zirconium, titanium, boron, and silver in order to improve the glass functionality and bioactivity. However, it is usually difficult to synthesize bioactive glasses in nano-size scale with addition of those ions. More recently, Delben et al. [52] have developed sol-gel derived bioactive glass doped with silver with a mean particle

size of 100 nm. It was reported that the Si-O-Si bond number increased with increasing silver concentration and this resulted in structural densification. It was also observed that quartz and metallic silver crystallization increased with the increase in silver content in bioactive glass while hydroxyapatite crystallization decreased [52].

Sol-gel derived bioactive glass nanoparticles have also been used to coat different materials to combine good mechanical properties and high bioactivity in one material [53,54]. Bioactive glass nanoparticle coating by sol-gel technique has been applied for example on the struts of porous HA by Esfahani et al. [53] in order to improve the mechanical properties of the scaffold. It was shown that the compressive strength of scaffolds increased and a new crystalline phase was detected with the increase in sintering temperature. According to Esfahani et al. [53] crystallization occurred in bioactive glass nanoparticles resulting in an improvement of the mechanical properties of the scaffolds.

For some applications, for example in tissue engineering scaffolds, it is advantageous to use anisotropic structures such as elongated particles or fibers. For this purpose, combinations of the sol-gel and electrospinning techniques have been developed [55]. Additives such as polyvinyl butyral are necessary to adjust the rheological properties of the sol for electrospinning. Similarly to conventional sol-gel synthesis, sol-gel derived and electrospun fibers must be submitted to heat-treatment to remove organic additives. Electrospinning of sol-gel precursors can result in bioactive glass fibers with diameters < 100 nm [55-57]. The diameter and the morphology of the nanofibers can be controlled to some extent by the amount and type of additive and the applied electric field. The resulting nanofibers, which are usually collected as mats, are flexible (due to their small diameter) and can be shaped into different morphologies being thus very attractive for tissue engineering scaffolds. However, individual fibres become fragile once immersed in simulated body fluid or when subjected to mineralization. Figure 4 shows scanning electron microscopy images of bioactive glass nanofibers prepared by electrospinning of a silicate sol, according to Xia et al. [56]. The distribution of the fiber diameter was reported to be between 50 and 110 nm, with average diameter 85 nm. More recently, Lu et al. [57] have developed bioactive glass nanofibers in the CaO-SiO₂ system (70S30C, 70 mol% SiO₂, 30 mol% CaO) by electrospinning method. A highly porous microstructure with interconnected

macropores and mesopores was obtained in the nanofibrous scaffold (Figure 5). The mechanical properties of the scaffolds obtained from 70S30C nanofibers were measured by nanoindentation. It was found that the elastic modulus of the scaffold was close to that of trabecular bone [57].

There is wide agreement about the versatility of the sol-gel technique to synthesize inorganic materials and it has been shown to be suitable for production of a variety of nanoscale bioactive glasses, as discussed in this section. However, the method is also limited in terms of compositions that can be produced. Moreover remaining water or residual solvent content may result in complications of the method for the intended biomedical applications of the nanoparticles or nanofibres produced. Usually a high temperature calcination step is required to eliminate organics remnants. In addition, sol-gel processing is relatively time consuming and since it is not a continuous process, batch-to-batch variations may occur.

3.2 Microemulsion techniques

Microemulsion has been known as a suitable technique able to obtain inorganic particles with particle size in the range of nanometers with minimum agglomeration [58]. Nanoparticles of oxides and carbonates have been successfully synthesized by microemulsion techniques [59-62]. A microemulsion is a thermodynamically stable transparent, isotropic dispersion of two immiscible liquids such as water and oil stabilized by surfactant molecules at the water/oil interface. In water-in-oil microemulsions, nanosized water droplets are dispersed in the continuous hydrocarbon phase and surrounded by the monolayer of surfactant molecules [63]. The size of the aqueous droplets is usually in the range 5 to 20 nm in diameter [61, 64]. These aqueous droplets act as a microreactor or nanoreactor in which reactions can take place when droplets containing the suitable reactants collide with each other. Precursor particles of hydroxide or oxalate are first formed in a microemulsion system. After drying and calcination of the precursor powder at an appropriate temperature, the desired oxide system is obtained. Microemulsion techniques are thus capable of delivering nanosized particles of organic and inorganic composition with minimum agglomeration since the reaction is taking place in nanosized domains. However, the main disadvantages of the

microemulsion technique are the low production yield and the usage of a large amount of oil and surfactant phases. Although microemulsion techniques provide an alternative way to other production methods for synthesizing several types of inorganic and organic nanosized particles [61, 65, 66], to the authors' knowledge, only few reports are available on the synthesis of nanosized bioactive glass particles by this method. Zhao et al. [67] for example synthesized bioactive nanoparticles in the system $\text{CaO-P}_2\text{O}_5\text{-SiO}_2$ by microemulsion method for bone tissue engineering scaffolds. Spherical amorphous particles were obtained with size in the 25-50 nm range. They reported that the diameter of the nanoparticles was related to the molar ratio of water to surfactant (γ) in water/oil emulsions. Water droplets were enlarged with the increase in the molar ratio of water to surfactant [67].

3.3. Gas phase synthesis method (flame spray synthesis)

Gas phase synthesis uses metal-organic precursor compounds to produce nanoparticles at temperatures above 1000°C . The basic principle of all gas phase synthesis methods is the formation of molecular nuclei which is followed by condensation and coalescence inducing the subsequent growth of nanoparticles in high temperature regions during the process. The most decisive factor for the final particle size is the mean residence time of the particles in the high temperature regions. High cooling rates ($>1000 \text{ K s}^{-1}$) and short residence times (1 ms) enable the nanoparticle formation. In contrast to wet phase processes, gas phase synthesis allows generally higher production rates. One of the most successful gas phase synthesis methods is flame spray synthesis which is a well known process and it is applied since 1940. It was originally developed for manufacturing carbon black [68] and is nowadays used to produce megatons of silica and titania nanoparticles per year. An advantage of this process in comparison to other gas phase processes is that no additional source of energy for precursor conversion such as plasma, lasers or electrically heated walls is required. An adaptation of the process allowing the use of organic liquid precursors loaded with metals instead of gaseous precursors proved to be very successful [69-71]. In this process, the liquid precursor is dispersed by oxygen over a nozzle thereby

forming a spray which is ignited. As the spray is burning, the organic constituents of the liquid precursor completely combust mainly to water and carbon dioxide and metal constituents oxidize to form the nanoparticles.

Several investigations have been carried out related to the flame spray process dynamics and there is understanding of the key variables involved and how they can be controlled to obtain nanoparticles of given size range and chemical composition [69-72]. It has been shown that the metal carboxylate system is a very convenient precursor because it allows the synthesis of oxide nanoparticles of almost any composition [71]. In addition, metal-organic salts are highly stable in air, tolerate humidity and most importantly they are fully miscible among each other. Consequently, the process allows the production of any kind of nanoparticulate mixed-oxides with high chemical homogeneity. Moreover, and depending on the composition, fast quenching after formation of the nanoparticles can preserve the amorphous state of the material [73, 74]. By using flame spray synthesis, therefore, the preparation of nanoparticles of different bioactive glass compositions has become possible. Mixtures of 2-ethylhexanoic acid salts of calcium and sodium, hexamethyldisiloxane, tributyl phosphate and fluorobenzene to introduce fluorine have been employed [73]. Figure 6 shows a schematic diagram of the flame spray synthesis process along with electron microscopy images of nanoparticulate bioactive glass prepared by flame spray synthesis [73- 75]. Furthermore, bismuth-2-ethylhexanoate has been used to add bismuth and to render bioactive glass nanoparticles radio-opaque [76]. As a result of the process characteristics and parameters, the primary particles produced are spherically shaped with different degrees of agglomeration (see Figure 6). Primary particles have been shown to exhibit a log normal particle size distribution [77].

3.4 Laser spinning technique

Laser spinning has been developed for the production of glass fibres with diameters in the nanometre to micrometre scale [78]. In laser spinning technique, large quantities of nanofibres can be produced with specific, controllable chemical compositions without the necessity of any chemical additives or post heat treatments. The process is very fast; nanofibres are produced in several microseconds. The laser spinning

technique essentially involves the quick heating and melting of a small volume of the precursor material up to high temperatures using a high power laser. At the same time, a supersonic gas jet is injected into the melt volume to blow the molten material [78-80]. Following this, the molten material is quickly stretched and cooled by the supersonic gas jet [81]. Long fibres with extraordinary high length to diameter ratios can be produced by the elongation process of the viscous molten material. The obtained material is in amorphous form because of the high cooling speed. Quintero et al. [78] used the laser spinning technique for the production of glass fibres in different compositions. They developed glass fibres in the form of a disordered net of intertwined amorphous micro- and nanofibres. Several starting materials with different compositions and microstructures such as soda-lime silicate glasses, polycrystalline ceramics and natural rocks were successfully used to produce glass fibres. Following studies showed the capability of the laser spinning technique to synthesize very long fibres at high speeds under ambient conditions [79, 80]. More recently, Quintero et al. [81] developed, to the authors' knowledge, the first bioactive glass nanofibers by the laser spinning technique. They produced bioactive glass nanofibers in the 45S5 Bioglass® and 52S4.6 compositions and investigated the possibility of using these nanofibers as scaffolds or as reinforcement in polymeric matrix composites. Disordered meshes of intertwined fibers with average diameter in the range 200–300 nm were obtained. The amorphous structure of the produced glass nanofibers was investigated by means of transmission electron microscopy (TEM). It was found that microstructure and composition of the precursor material had no influence on the amorphous structure of the fibers. The composition of the produced fibers was determined by means of both X-ray fluorescence (XRF) and magic angle spinning NMR (MAS-NMR) in order to determine whether or not they maintained the same chemical composition as the precursor glass. The results indicated that there were slight differences in compositions between bulk and fiber glasses. Bioactivity test results showed that the nanofibers were covered by a foamy and porous layer of amorphous calcium phosphate after immersion in SBF for 5 days. Energy dispersive X-ray spectroscopy (EDS) and selected area electron diffraction (SAED) TEM analyses also confirmed the bioactivity of the produced glass nanofibers. Consequently, the laser spinning technique was demonstrated to be a very effective method to produce bioactive glass nanofibers in

desired compositions and this novel technique represents a promising alternative for the fabrication of nanofibers to be used in polymer nanocomposites [81].

4. Composites containing nanoscaled bioactive glass

4.1 General characteristics of bioactive nanocomposites

The combination of biodegradable polymers and bioactive ceramics (and glasses) results in a new group of composite materials for applications as temporary orthopedic implants, bone filler materials or as 3D biocompatible scaffolds in the field of tissue engineering [12, 38]. The goal of these composite materials is to impart strength and bioactivity by an inorganic bioactive filler while keeping the positive properties of the polymer such as flexibility and capacity to deform under loads. The application of this class of composites in tissue engineering has been reviewed [12, 38].

Inorganic phases can be added to different polymer matrices in the form of micron sized or nanoscale particles or fibres. The size of the filler particles is an important parameter that affects the effective mechanical properties of composite materials. This is due to marked microstructural differences introduced by the micron sized or nanoscale fillers that contribute towards different interactions between the filler particles and the polymer matrix. For example, the introduction of nanoscale fillers with desired morphology usually increases the mechanical strength and stiffness of composites in comparison to the properties of the neat polymer and of composites with micron-size reinforcement [82]. The use of nanoscale degradable fillers such as bioactive glass or calcium phosphate nanoparticles should lead therefore to improved orthopedic implants and tissue engineering scaffolds. Additionally, in the case of bioactive silicate glass nanoparticles, they can produce a higher alkalinity when compared to commercially available (μm -sized) 45S5 Bioglass® [83]. This effect could buffer to a greater extent the acidic degradation of some polymers, e.g. polylactic acid, when nanoscale bioactive glass particles are used as filler in a composite.

The larger specific surface area of the nanoparticles should lead also to increased interface effects and it should contribute to improved bioactivity, when compared to

standard (μm -sized) particles. Moreover, the use of nanoparticles in a polymeric matrix mimics more closely the structure of natural bone, which contains nanoscale hydroxyapatite crystallites combined with the polymeric phase of collagen, being responsible for the desirable mechanical properties of bone. In this context, Webster et al. [84] have reported that a significant increase in protein adsorption and osteoblast adhesion has been observed on nanoscale ceramic materials compared to micron-sized ceramic materials and composites. Related results were achieved by Loher et al. [85] who demonstrated that bioactivity, degradation rate and mechanical properties of PLGA doped with nanoscale amorphous calcium phosphate are strongly improved with addition of nanoscale amorphous calcium phosphate particles when compared to the pure polymer [85].

In the following sections, a review of nanocomposite systems comprising nanoscale bioactive glass (nanoparticles or nanofibres) and biodegradable polymers is presented, focusing mainly on materials developed for bone regeneration strategies and tissue engineering.

4.2 Poly(3hydroxybutyrate) (P(3HB))/nanoparticulate bioactive glass composites

Recently, Misra et al. [86] have described the successful preparation of poly(3hydroxybutyrate) (P(3HB))/nanoparticulate bioactive glass composites with different filler concentrations by solvent casting. The thermal, mechanical and microstructural properties of these new composites were compared to their counterpart fabricated with micron-sized bioactive glass. Similarly to other studies in the literature [85], the addition of nanoparticles was shown to have a significant stiffening effect on the composite modulus, as shown in Figure 7.

It has been also shown [86], that systematic addition of bioactive glass nanoparticles induced a nanostructured topography on the surface of the composites, which was not visible on their micron-sized bioactive glass particle containing counterparts. This surface effect induced by the nanoparticles considerably improved total protein adsorption compared to the unfilled polymer and the composites containing micron-sized bioactive glass particles. An *in vitro* degradation study (30 days) in simulated body fluid (SBF) showed a high level of bioactivity as well as higher water absorption

for the nanoparticle containing composites. Furthermore, a preliminary cell proliferation study using osteoblast-like cells demonstrated the good cytocompatibility of the P(3HB)/bioactive glass composite systems [86]. Misra et al. [87] have also investigated in detail the effect of the addition of bioactive glass nanoparticles on the bioactivity, degradation and in vitro cytocompatibility of P(3HB)/nanoparticulate bioactive glass composites prepared by solvent casting technique. It was reported [87] that the ALP activity of MG-63 cells on nanoparticulate bioactive glass/P(3HB) composites was considerably higher than on the control surface. SEM micrographs of MG-63 cells attached on the surfaces of P(3HB) composites in Figure 8 show the cell morphology and the cell attachment to the substrates between days 4 and 7. There were no visible qualitative differences in the attachment of cells between the neat polymeric and composite samples. The cytocompatibility study (cell proliferation, cell attachment, alkaline phosphatase activity and osteocalcin production) using human MG-63 osteoblast-like cells in osteogenic and non-osteogenic medium showed the superiority of the composite substrates containing bioactive glass nanoparticles for the intended application in tissue engineering [87].

Zheng et al [88] have used another member of the PHA family, i.e. poly(hydroxybutyrate-2-co-2-hydroxyvalerate) (PHBV), to develop porous nanocomposites combining the polymer with biomimetically synthesized nano-sized bioactive glass (BMBG) particles in the system $\text{CaO-P}_2\text{O}_5\text{-SiO}_2$. Figures 9a,b are SEM images of the pore structure of the developed composites. The authors reported porosities > 90% indicating that the composite contained a great amount of interconnected pores [88]. The composites were shown to be bioactive as hydroxyl-carbonate-apatite (HCA) formed on the surface of specimens immersed in SBF for 8 hours (Fig. 9c) and further HCA development occurred after 24 hours in SBF (Fig. 9d). The study of cell attachment on the porous PHBV/BMBG composite indicated that the material has satisfactory bioactivity, bio-mineralization function and cell biocompatibility [88].

4.3 Poly(L-lactic acid) (PLLA)/bioactive glass nanocomposites

Hong et al. [42] have investigated a new family of composites combining poly(L-lactic acid) as biodegradable polymer and sol-gel derived bioactive glass-ceramic

(BGC) nanoparticles. 3D porous scaffolds were prepared by thermally-induced phase-separation combining poly(L-lactic acid) and different concentrations of BGC nanoparticles. The representative structure and porosity of such foams are depicted in Figure 10. The *in vitro* studies showed that composites containing BGC nanoparticles with lower phosphorous and higher silicon content have better bioactivity than that of the BGC with lower silicon and higher phosphorous content [42]. Hong et al. [89] have also studied the effect of nanoparticulate bioactive glass-ceramic content on the properties of nanocomposite scaffolds, in which an improvement of the mechanical properties could be detected. More recently, El-Kady et al. [90] have developed sol-gel derived bioactive glass nanoparticles/poly(L-lactide) (PLA) composites by using solid-liquid phase separation method combined with solvent extraction. They used a modified alkali-mediated sol-gel route to obtain bioactive glass nanoparticles. The modified sol-gel method resulted in reduction of the gelation time to about a minute rather than days as in the traditional sol-gel process. Furthermore, fast gelation prevented the aggregation and growth of colloidal particles to sizes larger than 100 nm. The proposed method [90] is thus capable of delivering nanoparticles of sizes less than 100 nm with minimum agglomeration. It was reported that the scaffold's pore size decreased with the increase in the glass nanoparticles content. The *in vitro* studies revealed that the addition of bioactive glass nanoparticles improved the bioactivity of the scaffolds [90].

During the preparation of this type of nanocomposites, it is possible that nanoparticles aggregate in the matrix because of their incompatibility with the biopolymer used, resulting in a deterioration of the composite mechanical properties. A new approach has been reported by Liu et al. [91, 92] to improve the mechanical properties of nanoparticulate bioactive glass/PLLA composites. It was shown that surface modification of nano-sized bioactive glass particles by grafting organic molecules or polymers is a convenient solution to improve the mechanical properties of the composites. The modification induces the formation of a buffer layer between the nanoparticulate bioactive glass and the polymer matrix, which improves the dispersion of the nano-sized particles within the matrix without any agglomeration. This results in a significant improvement of the final mechanical properties of the composite materials [91, 92]. Liu et al. [91] developed surface modified bioactive glass nanoparticles/poly(L-lactide) (PLLA) composites by using solvent evaporation

technique. Low-molecular-weight PLLA was grafted onto the surface of the sol-gel derived bioactive glass nano-particles by diisocyanate and the ring-opening polymerization of the L-lactide [92]. They reported that the mechanical properties of the surface modified bioactive glass/PLLA composites were better than those of the non-modified bioactive glass/PLLA composites [91, 92]. The morphology of fracture surfaces of composites containing modified and non-modified bioactive glass nanoparticles were compared and linked to the different fracture properties of the composites. It was reported that the roughness of fracture surfaces of composites with modified nanoparticles decreased compared with the non-modified ones. For example, Figure 11 shows SEM micrographs of fracture surfaces of PLLA/bioactive glass nanocomposites containing modified and non-modified nanoparticles in two different concentrations (4 wt.% and 20 wt.%) [91]. Nanoparticle aggregation in composites with modified nanoparticles was not observed in contrast to composites containing non-modified bioactive glass particles, due to the improvement of the phase compatibility between the modified nanoparticles and PLLA matrix. Furthermore, the surface modified bioactive glass nanoparticles were seen to act as nucleation sites improving the degree of crystallization of the matrix. The composites were shown to be bioactive as a calcium phosphate layer formed on the surfaces upon immersion in SBF. It was also demonstrated that surface modified bioactive glass/PLLA composites exhibited much better cell proliferation ability than non-modified bioactive glass/PLLA composites and pure PLLA [91, 92].

4.4 Natural polymer / bioactive glass nanocomposites

Besides synthetic polymers discussed above, natural-based materials such as polysaccharides (starch, chitin, chitosan) or proteins (silk, collagen) can be used as polymer matrices to prepare nanocomposites. Peter et al. [93, 94] have synthesized α -chitin/sol-gel derived bioactive glass ceramic nanoparticle and chitosan/ sol-gel derived bioactive glass ceramic nanoparticle composite scaffolds by using lyophilization technique. They developed macroporous composite scaffolds with pore size in the range 150-500 μm [94] The developed composite scaffolds demonstrated adequate swelling and degradation with the addition of nano-sized bioactive glass-ceramic particles. In

vitro studies showed the deposition of apatite on the surface of the composite scaffolds, indicating the bioactive nature of the composite scaffolds. The investigation of the in vitro behaviour considering osteoblast-like cells (MG-63) indicated that cells attached on the pore walls of the scaffolds and showed initial signs of spreading [93, 94].

Wang et al.[95] developed a new porous bioactive nanocomposite composed of sol-gel derived bioactive glass nanoparticles (BG), collagen (COL), hyaluronic acid (HYA) and phosphatidylserine (PS) by a combination of sol-gel and freeze-drying methods. They also synthesized a bioactive nanocomposite by crosslinking collagen and HYA by using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS). After crosslinking, the structure of BG-COL-HYA-PS scaffolds became more ordered and channel pores preferentially aligned. The scaffolds were seen to be highly porous with pore size in the range 100-400 μm . It was reported that biomineralization and degradation in SBF, and mechanical strength of the EDC/NHS-crosslinked BG-COL-HA-PS composite scaffolds were better than those of the scaffolds without HYA, PS, and crosslinking process. PS and HYA play an important role in the regulation of the biomineralization process, inducing HA to precipitate on the surface of the composites. Further *in vitro* cell culture studies demonstrated that MC3T3-E1 cells attached and spread on the surface of crosslinked BG-COLHYA-PS scaffolds indicating the biocompatibility of the nanocomposite [95]. Xie et al. [96] have investigated the *in vivo* bone regeneration ability of the EDC/NHS-crosslinked BG-COL-HA-PS composite scaffolds using a rabbit radius defect model. After implantation, radiological, histological and micro-CT studies were conducted at 2, 4 and 8 weeks. Ectopic bone formation was also investigated in a rat model. X-ray and histological studies showed the ability of bone regeneration for both nanocomposites and for nanocomposites combined with growth factors (BMP). However, the bone defect was covered with new bone only in the nanocomposites grafted with BMP at 8 weeks. Moreover, the nanocomposite combined with BMP showed a better ability of ectopic bone formation compared with the composites without BMP [96].

More recently, Couto et al. [97] have developed chitosan and bioactive glass nanoparticle multilayer coatings by a well developed sequential deposition method, also known as layer-by-layer (LbL) technique. SEM observations revealed that the spherical nanoparticles with sizes that varied from 30 to 100 nm homogeneously dispersed on the

surface of the multilayered coatings. Chitosan provided viscoelastic properties to the final coating, while the bioactive glass provided bioactivity for the organic-inorganic structure. *In vitro* studies indicated that the conceived multilayers induced the formation of apatite on a marker of bioactive behavior. This work clearly showed that LbL technique can be applied to coat different prosthetic devices for orthopaedic application or scaffolds for bone tissue engineering [97].

4.5 Bioactive nanocomposites containing bioactive glass nanofibres

Recently, a series of composites of various morphologies, such as fibrous membranes and 3D porous scaffolds, are being developed by compounding polymers and bioactive glass nanofibre (BGNF). Kim et al. [98] were the first to develop a composite of PLA filled with sol-gel-derived bioactive glass as a nanoscale composite fiber by means of electrospinning (ES). Nanocomposites with a dense nanofibrous network were achieved. It was observed that glass nanofibers were uniformly dispersed in the PLA matrix [98]. The *in vitro* bioactivity and osteoblast responses of the developed nanocomposites were also studied by Kim et al. [99]. The nanocomposites showed excellent bioactivity, inducing CaP precipitation within 24 h of immersion in SBF. It was also reported that the osteoblast response of the nanocomposites was significantly improved as the amount of bioactive nanofibers increased [99]. Kim et al. [100] also developed BGNF-collagen nanocomposite both in the form of a thin membrane and as macroporous scaffold. SEM investigations revealed the similar composite microstructure of both membranes and porous scaffolds with uniformly distributed BGNF in the collagen matrix (Figure 12). TEM studies showed that both BGNF and collagen were in the nanoscale. BGNF-collagen nanocomposites exhibited high bioactivity, assessed by the rapid formation of bone-like apatite minerals on their surfaces when immersed in SBF. It was also observed that the nanocomposites assisted the adhesion and growth of human osteoblast-like cells *in vitro* [100].

Lee et al. [101] produced poly(*ε*-caprolactone) (PCL)/sol-gel derived BGNF nanocomposite in a thin membrane form. The glass nanofibrous were distributed well within the PLC matrix, showing a much rougher surface than the pure PCL. *In vitro* studies showed that the precipitated apatite covered the surface of the nanocomposite

membrane almost completely after immersion in SBF for 14 days. Osteoblastic cells (MC3T3-E1) on the nanocomposite membrane spread better and grew actively with many cytoplasmic extensions, showing improved proliferation behavior than those on the pure PCL membrane [101]. More recently, Jo et al. [102] have fabricated (PCL)/sol-gel derived BGNF composites and investigated their biocompatibility and mechanical properties in comparison with composites containing the microparticulate form of bioactive glass. Nano-sized bioactive glass fibers were uniformly distributed in the polymer matrix as a result of their uniform shape and size, in contrast to the micron-sized bioactive glass fibers. This microstructure resulted in a significant improvement of the biological and mechanical properties of the PCL/BGNF composites, compared to that of the micron-sized ones. In Figure 13, the elastic modulus of the PCL/BGP and PCL/BGNF composites are compared with those of the PCL control, indicating the superior elastic modulus of the nanocomposites. Furthermore, *in vivo* animal test results revealed the good biocompatibility of the PCL/BGNF composite and its bone-forming ability was demonstrated when implanted in a calvarial bone defect [102].

The introduction of bioactive glass nanofibres as filler in biodegradable polymers adds therefore interesting features and represents a promising step towards the development of improved biomaterials for bone regeneration as well as engineered scaffolds for tissue engineering applications. More research is indeed required to exploit the novel properties of these composites, in different morphologies, for a variety of applications in hard tissue regeneration and bone tissue engineering.

5. Conclusions

The preparation of bioactive glasses in nanoparticle and nanofibre form has recently become feasible by advances in wet and dry synthesis methods. Nanoscale particulate and nanofibre bioactive glasses have shown advantages over conventional (micron-sized) bioactive glasses due to their large surface area and enhanced solubility as well as reactivity coupled with the possibility to induce nanotopographic surface features in composite materials. These nanomaterials have also inspired researchers to investigate new applications of bioactive glasses in biomedical engineering. Their clinical effectiveness, however, still needs to be tested and fully validated in *in vivo*

experiments. The great potential of nanometric bioactive glass systems lies not only in the field of bone tissue engineering but also in dentistry, for example in dentin regeneration and in the reconstruction of critical bone defects as well as in osteochondral and cartilage regeneration. The works reviewed in the present paper show that the development of composite materials combining biodegradable polymers (synthetic and natural) with nanoscale bioactive glass particles or fibres is emerging as a powerful approach toward 3rd generation bioactive materials and the biomedical applications of these novel materials are bound to expand. Substantial advantages of these systems compared to conventional (μm -scale) bioactive glass containing composites are being demonstrated, as review in this paper. The possibility of processing such inorganic nanostructured bioactive materials will also permit to use them in more sophisticated concepts such as in the spatial control at the micro/nano-levels of bioactivity of surfaces, injectable osteoconductive biomaterials, thin coatings and films or self-assembling osteoconductive nanobiomaterials.

6. References

1. Hench LL, Splinter RJ, Allen WC, Greenlee TK. Bonding mechanisms at the interface of ceramic prosthetic materials. *J Biomed Mater Res* 1971;5(6):117-141.
2. Hench LL. Bioceramics. *J Am Ceram Soc* 1998;81(7):1705-1728.
3. Sepulveda P, Jones JR, Hench LL. Characterization of melt-derived 45S5 and sol-gel-derived 58S bioactive glasses. *J Biomed Mater Res* 2001;58(6):734-740.
4. Saravanapavan P, Jones JR, Pryce RS, Hench LL. Bioactivity of gel-glass powders in the CaO-SiO₂ system: A comparison with ternary (CaO-P₂O₅-SiO₂) and quaternary glasses (SiO₂-CaO-P₂O₅-Na₂O). *J Biomed Mater Res A* 2003;66A(1):110-119.
5. Oki A, Parveen B, Hossain S, Adeniji S, Donahue H. Preparation and in vitro bioactivity of zinc containing sol-gel-derived bioglass materials. *J Biomed Mater Res A* 2004;69A(2):216-221.

6. Vallet-Regi M, Salinas AJ, Roman J, Gil M. Effect of magnesium content on the in vitro bioactivity of CaO-MgO-SiO₂-P₂O₅ sol-gel glasses. *J Mater Chem* 1999;9(2):515-518.
7. Liu X, Huang W, Fu H, Yao A, Wang D, Pan H, Lu WW, Jiang, X, Zhang X, Bioactive borosilicate glass scaffolds: in vitro degradation and bioactivity behaviours, *J. Mater. Sci. Mater. Med.* 2009;20:1237-1243.
8. Lao J, Nedelec JM, Jallot E, New strontium-based bioactive glasses: physicochemical reactivity and delivering capability of biologically active dissolution products, *J. Mater. Chem.* 2009;19:2940-2949.
9. Varanasi VG, Saiz E, Loomer PM, Ancheta B, Uritani N, Ho SP, Tomsia AP, Marshall SJ, Marshall GW, Enhanced osteocalcin expression by osteoblast-like cells (MC3T3-E1) exposed to bioactive coating glass (SiO₂-CaO-P₂O₅-MgO-K₂O-Na₂O system) ions, *Acta Biomater* 2009;5:3536-3547.
10. Li R, Clark AE, Hench LL. An Investigation of Bioactive Glass Powders by Sol-Gel Processing. *J App Biomater* 1991;2(4):231-239.
11. Ducheyne P, Bioglass coatings and bioglass composites as implant materials, *J. Biomed. Mater. Res.* 1985; 19:273-291.
12. Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. *Biomaterials* 2006;27(18):3413-3431.
13. Zamet JS, Darbar UR, Griffiths GS, Burgin W, Newman HN. Particulate bioglass (Perioglas(R)) in the treatment of periodontal intrabony defects. *J Dent Res* 1997;76:2219-2219.
14. Gatti, AM, Simonetti LA, Monari E, Guidi S, Greenspan D. Bone augmentation with bioactive glass in three cases of dental implant placement. *J Biomater Appl* 2006;20(4):325-339.
15. Zehnder M, Soderling E, Salonen J, Waltimo T. Preliminary evaluation of bioactive glass S53P4 as an endodontic medication in vitro. *J Endodont*, 2004;30(4):220-224.
16. Waltimo T, Mohn D, Paque F, Brunner TJ, Stark W J, Imfeld T, Schaetzle M, Zehnder M. Fine-tuning of Bioactive Glass for Root Canal Disinfection, *J Dent Res* 2009;88(3):235-238.

17. Gomez-Vega JM, Saiz E, Tomsia AP, Marshall GW, Marshall SJ. Bioactive glass coatings with hydroxyapatite and Bioglass (R) particles on Ti-based implants. 1. Processing. *Biomaterials* 2000;21(2):105-111.
18. Kitsugi T, Nakamura T, Oka M, Senaha Y, Goto T, Shibuya T. Bone-bonding behavior of plasma-sprayed coatings of Bioglass(R), AW-glass ceramic, and tricalcium phosphate on titanium alloy. *J Biomed Mater Res* 1996;30(2):261-269.
19. Xynos ID, Hukkanen MVJ, Batten JJ, Buttery LD, Hench LL, Polak JM. Bioglass (R) 45S5 stimulates osteoblast turnover and enhances bone formation in vitro: Implications and applications for bone tissue engineering. *Calcified Tissue Int* 2000;67(4):321-329.
20. Yao J, Radin S, Leboy S, Ducheyne P. The effect of bioactive glass content on synthesis and bioactivity of composite poly(lactic-co-glycolic acid)/bioactive glass substrate for tissue engineering. *Biomaterials* 2005;26(14):1935-1945.
21. Hench LL Polak JM. Third-generation biomedical materials. *Science* 2002;295(5557):1014-1017.
22. Chen QZZ, Thompson ID, Boccaccini AR. 45S5 Bioglass (R)-derived glass-ceramic scaffolds for bone tissue engineering. *Biomaterials* 2006;27(11):2414-2425.
23. Mano JF, Sousa RA, Boesel LF, Neves NM, Reis RL. Bioinert, biodegradable and injectable polymeric matrix composites for hard tissue replacement: state of the art and recent developments. *Comp Sci Tech* 2004; 64(6): 789-817.
24. Shi J, Alves NM, Mano JF. Smart temperature-responsive biomineralization onto biodegradable polymeric substrates. *Adv Funct Mater* 2007;17(16):3312-3318.
25. Dias CI, Mano JF, Alves NM. pH responsive biomineralization onto chitosan grafted biodegradable substrates. *J Mater Chem* 2008;18(21):2493-2499.
26. Wheeler DL, Montfort MJ, McLoughlin SW. Differential healing response of bone adjacent to porous implants coated with hydroxyapatite and 45S5 bioactive glass, *J. Biomed. Mater. Res.* 2001;55: 603-612.

27. Day, R. M., Boccaccini, A. R., Shurey, S., Roether, J. A., Forbes, A., Hench, L. L., Gabe, S. M. Assessment of polyglycolic acid mesh and bioactive glass for soft-tissue engineering scaffolds, *Biomaterials* 2004;25:5857-5866.
28. Gorustovich AA, Roether JA, Boccaccini AR, Effect of Bioactive Glasses on Angiogenesis: In-vitro and In-vivo Evidence. A Review, *Tissue Eng. Part B* (in press, 2010).
29. Leu A, Leach JK. Proangiogenic potential of a collagen/bioactive glass substrate. *Pharm Res* 2008;25:1222-1229.
30. Bellantone M., Williams H.D., Hench L.L. Broad-spectrum bactericidal activity of Ag₂O-doped bioactive glass, *Antimicrob Agents Chemother*, 2002;46: 1940-5.
31. Vitale-Brovarone C, Miola M, Balagna C, Verne E, 3D-glass-ceramic scaffolds with antibacterial properties for bone grafting, *Chemical Eng. J.* 2008;137:129-136.
32. Haimi S, Gorianc G, Moimas L, Lindroos B, Huthala H, et al, Characterization of zinc-releasing three dimensional bioactive glass scaffolds and their effect on human adipose stem cell proliferation and osteogenic differentiation, *Acta Biomater.* 2009; 5:3122-3131
33. Azevedo M, Jell G, Hill R. Stevens MM, Novel hypoxia mimicking bioactive materials for tissue engineering, *Tissue Eng. A* 2008;: 14:889-889 (Meeting Abstract: P288)
34. Ostomel TA, Shi Q, Tsung CK, Liang H, Stucky GD. Spherical bioactive glass with enhanced rates of hydroxyapatite deposition and hemostatic activity, *Small* 2006; 2(11):1261-5.
35. Bretcanu O, Spriano S, Vitale CB, Verne E, Synthesis and characterization of coprecipitation-derived ferrimagnetic glass-ceramic, *J. Mater. Sci.* 2006;41: 1029-1037.
36. Kay, S., Thapa, A., Haberstroh, K.M., and Webster, T.J., Nanostructured polymer/nanophase ceramic composites enhance osteoblast and chondrocyte adhesion. *Tissue Eng*, 2002;8:753-761.

37. Palin E, Liu HN, Webster TJ. Mimicking the nanofeatures of bone increases bone-forming cell adhesion and proliferation. *Nanotechnology*, 2005;16(9):1828-1835.
38. Guarino V, Causa F, Ambrosio L. Bioactive scaffolds for bone and ligament tissue. *Expert Rev Medical Devices* 2007;4(3): 405-418.
39. Alves NM, Leonor IB, Azevedo HS, Reis RL, Mano JF. Designing biomaterials based on biomineralization of bone, submitted.
40. Scherrer GW, Brinker CJ. *Sol–Gel Science: The Physics and Chemistry of Sol-Gel*. 1990, Boston, MA: Academic Press.
41. Xia, W. and Chang, J., Preparation and characterization of nano-bioactive-glasses (NBG) by a quick alkali-mediated sol-gel method. *Mater Lett* 2007;61(14-15):3251-3253.
42. Hong Z, Reis RL, Mano JF. Preparation and in vitro characterization of novel bioactive glass ceramic nanoparticles. *J Biomed Mater Res A* 2009;88(2):304-313.
43. Huang DM, Hung Y, Ko BS, Hsu SC, Chen WH, Chien CL, Tsai CP, Kuo CT, Kang JC, Yang CS, Mou CY, Chen YC, Highly efficient cellular labeling of mesoporous nanoparticles in human mesenchymal stem cells: implication for stem cell tracking, *Faseb Journal* 2005;19: 2014.
44. Lin YS, Tsai CP, Huang HY, Kuo CT, Hung Y, Huang DM, Chen YC, Mou CY, Well-ordered mesoporous silica nanoparticles as cell markers, *Chem. Mater.* 2005;17: 4570-4573.
45. Yap FL, Zhang Y, Protein and cell micropatterning and its integration with micro/nanoparticles assembly, *Biosensors and Bioelectronics* 2007; 22:775-788.
46. Veerapandian M, Yun K, The state of the art in biomaterials as nanobiopharmaceuticals, *Digest J. Nanomater. Biostructures* 2009;4: 243-262
47. Hong Z, Liu A, Chen L, Chen X, Jing X. Preparation of bioactive glass ceramic nanoparticles by combination of sol-gel and coprecipitation method. *J Non-Cryst Solids* 2009;355(6):368-372.
48. Couto DS, Hong Z, Mano JF. Development of bioactive and biodegradable chitosan-based injectable systems containing bioactive glass nanoparticles. *Acta Biomater* 2009;5(1):115-123.

49. Hong Z, Merino EG, Reis RL, Mano JF. Novel Rice-shaped Bioactive Glass Ceramic Nanoparticles. *Adv Eng Mater* 2009;11(15):B25-29.
50. Chen X, Lei B, Wang Y, Zhao N. Morphological control and in vitro bioactivity of nanoscale bioactive glasses. *J Non-Cryst Solids* 2009;355: 791–796.
51. Chen X, Guo C, Zhao N. Preparation and characterization of the sol–gel nano-bioactive glasses modified by the coupling agent gamma-aminopropyltriethoxysilane. *Appl Surf Sci* 2008;255:466–468.
52. Delben JRJ, Pimentel OM, Coelho MB, Candelario PD, Furini LN, Santos FA, Vicente FS, Delben AAST. Synthesis and thermal properties of nanoparticles of bioactive glasses containing silver. *J Therm Anal Calorim* 2009;97:433–436.
53. Esfahani SIR, Tavangarian F, Emadi R. Nanostructured bioactive glass coating on porous hydroxyapatite scaffold for strength enhancement. *Mater Lett* 2008;62:3428–3430.
54. Fathi MH, Doostmohammadi A. Bioactive glass nanopowder and bioglass coating for biocompatibility improvement of metallic implant. *J Mater Process Tech* 2009;209:1385–1391.
55. Kim HW, Kim HE, Knowles JC. Production and potential of bioactive glass nanofibers as a next-generation biomaterial. *Adv Func Mater* 2006;16(12):1529-1535.
56. Xia W, Zhang DM, Chang J. Fabrication and in vitro biomineralization of bioactive glass (BG) nanofibres. *Nanotechnology* 2007;18:135601(7 pp).
57. Lu H, Zhang T, Wang XP, Fang QF. Electrospun submicron bioactive glass fibers for bone tissue scaffold. *J Mater Sci: Mater Med* 2009;20:793–798.
58. Pileni MP. The role of soft colloidal templates in controlling the size and shape of inorganic nanocrystals. *Nature Mater* 2003;2:145-150.
59. Sun Y, Guo G, Tao D, Wang Z. Reverse microemulsion-directed synthesis of hydroxyapatite nanoparticles under hydrothermal conditions. *J Phys Chem Solids* 2007;68:373-377
60. Singh S, Bhardwaj P, Singh V, Aggarwal S, Mandal UK. Synthesis of nanocrystalline calcium phosphate in microemulsion—effect of nature of surfactants. *J Colloid Interf Sci* 2008;319:322-329.

61. Lim GK, Wang J, Ng SC, Gan LM. Processing of fine hydroxyapatite powders via an inverse microemulsion route. *Mater Lett* 1996;28:431-436.
62. Karagiozov C, Momchilova D. Synthesis of nano-sized particles from metal carbonates by the method of reversed mycelles *Chem Eng Process* 2005;44:115-119.
63. Arriagada FJ. Synthesis of nanosize silica in a nonionic water-in-oil microemulsion. *J Colloid Interf Sci* 1999;211:210-220.
64. Paul BK, Moulik SP. Microemulsions: An overview. *J Disper Sci Technol* 1997;18(4):301-367.
65. Lim GK, Wang J, Ng SC, Gan LM. Formation of nanocrystalline hydroxyapatite in nonionic surfactant emulsions. *Langmuir* 1999;15:7472-7477.
66. Bose S, Saha SK. Synthesis and characterization of hydroxyapatite nanopowders by emulsion technique. *Chem Mater* 2003;15:4464-4469.
67. Zhao NR, Wang YJ, Chen XF, Yang YX, Wei K, Wu G. Preparation of bioactive nanoparticles in the system $\text{CaO-P}_2\text{O}_5\text{-SiO}_2$ using microemulsions. *Key Eng Mater* 2005;288-289:179-182.
68. Pratsinis S.E. Flame aerosol synthesis of ceramic powders. *Prog Energ Combust Sci* 1998;24(3):197-219.
69. Stark WJ, Madler L, Maciejewski M, Pratsinis SE, Baiker A. Flame synthesis of nanocrystalline ceria-zirconia: effect of carrier liquid. *Chem Commun* 2003;5:588-589.
70. Stark WJ, Mädler L, Pratsinis SE. Metal oxides prepared by flame spray pyrolysis. WO 2004/005184, 2004.
71. Stark WJ Pratsinis SE. Metal delivery system for nanoparticle manufacture. WO 2004/103900A1, 2004.
72. Athanassiou EK, Grass RN, Stark W J. Chemical aerosol engineering as a novel tool for material science: from oxides to salt and metal nanoparticles. *Aerosol Sci Tech* 2009, in press, DOI: 10.1080/02786820903449665
73. Brunner TJ, Grass RN, Stark WJ. Glass and bioglass nanopowders by flame synthesis. *Chem Commun* 2006;13:1384-1386.74.
74. Lohrer S, Stark WJ, Maciejewski M, Baiker A, Pratsinis SE, Reichardt D, Maspero F, Krumeich F, Günther D. Fluoro-apatite and calcium phosphate

- nanoparticles by flame synthesis. *Chem Mater* 2005;17(1):36-42.
- 75 Brunner, T. J., Stark, W. J., Boccaccini, A. R., *Nanoscale Bioactive Silicate Glasses in Biomedical Applications*, Chapter 6 in: *Nanomaterials for the Life Sciences Vol. 2: Nanostructured Oxides*, Challa S. S. R. Kumar, ed., (Wiley-VCH GmbH & Co. KGaA, Weinheim, Germany) 2009, pp. 203-220.
- 76 Mohn D, Zehnder M, Imfeld T, Stark WJ. Radio-opaque nanosized bioactive glass for potential root canal application: Evaluation of radiopacity, bioactivity, and alkaline capacity. *Int Endod J*, 2009, accepted.
- 77 Dekkers PJ Friedlander SK. The self-preserving size distribution theory I. Effects of the Knudsen number on aerosol agglomerate growth. *J Colloid Interf Sci* 2002;248(2):295-305.
- 78 Quintero F, Mann A B, Pou J, Lusquinos F, Riveiro A. *Appl Phys Lett* 2007;90:153109.
- 79 Quintero F, Pou J, Lusquinos F, Riveiro A. Experimental analysis of the production of micro- and nanofibres by Laser Spinning. *Appl Surf Sci* 2007;254:1042-1047.
- 80 Quintero F, Dieste O, Pou J, Lusquinos F, Riveiro A. On the conditions to produce micro- and nanofibres by laser spinning. *J Phys D: Appl Phys* 2009;42:065501(10pp).
- 81 Quintero F, Pou J, Comesana R, Lusquinos F, Riveiro A, Mann AB, Hill RG, Wu ZY, Jones JR. Laser spinning of bioactive glass nanofibers. *Adv Funct Mater* 2009;19;1-7.
- 82 Koo, JH. *Polymer Nanocomposites*, McGraw-Hill, USA (2006).
- 83 Vollenweider M, Brunner TJ, Knecht S, Grass RN, Zehnder M, Imfeld T, Stark WJ. Remineralization of human dentin using ultrafine bioactive glass particles. *Acta Biomaterialia* 2007;3(6):936-943.
- 84 Webster TJ, Siegel RW, Bizios R. Osteoblast adhesion on nanophase ceramics. *Biomaterials* 1999;20(13):1221-1227.
- 85 Loher S, Reboul V, Brunner TJ, Simonet M, Dora C, Neuenschwander P, Stark WJ. Improved degradation and bioactivity of amorphous aerosol derived tricalcium phosphate nanoparticles in poly(lactide-co-glycolide). *Nanotechnology* 2006;17(8):2054-2061.

86. Misra SK, Mohn D, Brunner TJ, Stark WJ, Philip SE, Roy I, Salih V, Knowles JC, Boccaccini AR. Comparison of nanoscale and microscale bioactive glass on the properties of P(3HB)/Bioglass(R) composites. *Biomaterials* 2008;29(12):1750-1761.
87. Misra SK, Ansari T, Mohn D, Valappil SP, Brunner TJ, Stark WJ, Roy I, Knowles JC, Sibbons PD, Jones EV, Boccaccini AR, Salih V. Effect of nanoparticulate bioactive glass particles on bioactivity and cytocompatibility of poly(3-hydroxybutyrate) composites. *J. Royal Soc. Interface* 2010; 7:453-465.
88. Zheng, H.D., Wang, Y. J., Yang, C. R., Chen, X. F., Zhao, N. R., Investigation on the Porous Biomaterial for Bone Reconstruction with Addition of Bio-mimetic Nano-Sized Inorganic Particles. *Key Eng Mater* 2007;336-338:1534-1537.
89. Hong Z, Reis RL, Mano JF. Preparation and in vitro characterization of scaffolds of poly(l-lactic acid) containing bioactive glass ceramic nanoparticles. *Acta Biomaterialia* 2008;4:1297-1306.
90. El-Kady AM, Ali AF, Farag MM. Development, characterization, and in vitro bioactivity studies of sol-gel bioactive glass/poly(L-lactide) nanocomposite scaffolds. *Mater Sci Eng C* 2010;30(1):120-131.
91. Liu A, Hong Z, Zhuang X, Chen X, Cui Y, Liu Y, Jing X. Surface modification of bioactive glass nanoparticles and the mechanical and biological properties of poly(L-lactide) composites. *Acta Biomaterialia* 2008;4(4):1005-1015.
92. Liu A, Wei J, Chen X, Jing X, Cui Y, Liu Y. Novel Composites Of Poly(L-Lactide) And Surface Modified Bioactive SiO₂-CaO-P₂O₅ Gel Nanoparticles: Mechanical And Biological Properties. *Chinese J Polym Sci* 2009;27(3):415-426.
93. Peter M, Kumar PTS, Binulal NS, Nair SV, Tamura H, Jayakumar R. Development of novel α -chitin/nanobioactive glass ceramic composite scaffolds for tissue engineering applications. *Carbohydr Polym* 2009;78(4):926-931.
94. Peter M, Binulal NS, Soumya S, Nair SV, Furuike T, Tamura H, Jayakumar R. Nanocomposite scaffolds of bioactive glass ceramic nanoparticles disseminated chitosan matrix for tissue engineering applications *Carbohydr Polym* 2010;79(2):284-289.

95. Wang Y, Yang C, Chen X, Zhao N. Development and characterization of novel biomimetic composite scaffolds based on bioglass-collagen-hyaluronicacid-phosphatidylserine for tissue engineering applications. *Macromol Mater Eng* 2006;291:254–262.
96. Xie E, Hu Y, Chen X, Bai J, Ren L, Zhang Z. A novel nanocomposite and its application in repairing bone defects. *Proceedings of the 3rd IEEE Int. Conf. on Nano/Micro Engineered and Molecular Systems*, Sanya, China: 2008. p.943-946,
97. Couto DS, Alves NM, Mano JF. Nanostructured Multilayer Coatings Combining Chitosan with Bioactive Glass Nanoparticles. *J Nanosci Nanotechno* 2009;9:1741–1748.
98. Kim HW, Kim HE, Knowles JC. Production and potential of bioactive glass nanofibers as a next-generation biomaterial. *Adv Funct Mater* 2006;16:1529–1535.
99. Kim HW, Lee HH, Chun GS. Bioactivity and osteoblast responses of novel biomedical nanocomposites of bioactive glass nanofiber filled poly(lactic acid). *J Biomed Mater Res A* 2008;85A(3):651-663.
100. Kim HW, Song JH, Kim HE. Bioactive glass nanofiber–collagen nanocomposite as a novel bone regeneration matrix. *J Biomed Mater Res A* 2006;79(3):698-705.
101. Lee HH, Yu HS, Jang JH, Kim HW. Bioactivity improvement of poly(ϵ -caprolactone) membrane with the addition of nanofibrous bioactive glass. *Acta Biomaterialia* 2008;4:622–629.
102. Jo JH, Lee EJ, Shin DS, Kim HE, Kim HW, Koh YH, Jang JH. In vitro/In vivo biocompatibility and mechanical properties of bioactive glass nanofiber and poly(ϵ -caprolactone) composite materials. *J Biomed Mater Res B: Appl Biomaterials* 2009;91B(1):213-220.

FIGURE CAPTIONS

Figure 1. Schematic diagram for the sol-gel synthesis process of bioactive glass nanoparticle. Drafted according to the methods described in ref. [42, 47].

Figure 2. SEM micrographs of bioactive glass nanoparticles with different shape and composition. (A) Spherical bioactive glass nanoparticles with the formulation $\text{SiO}:\text{P}_2\text{O}_5:\text{CaO} = 55:40:5$ (mol). (Reproduced from ref. [48] with permission of Elsevier). (B) Rice-shaped bioactive glass nanoparticles with the formulation $\text{SiO}:\text{P}_2\text{O}_5:\text{CaO} = 6:74:20$ (mol). Scale bars are 500nm.

Figure 3. TEM images of bioactive glass nanoparticles obtained without LA (A), with LA/TEOS:0.005 (mol%) (B), LA/TEOS:0.01 (mol%) (C), and with LA/TEOS:0.03 (mol%) (D). (Reprinted from ref. [50] with permission of Elsevier).

Figure 4. Scanning electron microscopy images of bioactive glass nanofibres prepared by electrospinning (A), after calcination at 600°C (B). (Reprinted from ref. [56] with permission of IOP Publishing Ltd, UK)

Figure 5. Typical SEM images of electrospun submicron bioactive glass 70S30C fibers at different magnification (a–c), and SEM image of a single fiber (d). (Reproduced from ref. [57] with permission of Springer).

Figure 6. Electron microscopy image of nanoparticulate bioactive glass (nominal composition 45S5 Bioglass®) as prepared by flame spray synthesis as well as a scheme representing the flame spray synthesis process. (Reproduced from ref.s [73,74] with permission of the American Chemical Society and The Royal Society of Chemistry, respectively)

Figure 7. Young's modulus of composites consisting of different concentrations of micron- or nano-sized bioactive glass particles in poly(3hydroxybutyrate) compared to the neat polymer [86].

Figure 8. SEM images showing MG-63 cells grown on (a) P(3HB) at day 4, (b) P(3HB) at day 7, (c) P(3HB)/20 wt % n-BG at day 4, and (d) P(3HB)/20 wt % n-BG at day 7. Scale bar, 100 μ m. [87] (Reproduced from ref. [87] with permission of the Royal Society, UK)

Figure 9. SEM images of PHBV/BMBG porous composites immersed in SBF for different times. (a) before immersion; (b) the locally magnified morphology of pore wall before immersion; (c) 8 hours immersion and (d) 24 hours immersion [88] (Reprinted from ref. [88] with permission of Trans Tech Publications)

Figure 10. Scanning electron micrographs of poly(L-lactic acid) scaffolds without bioactive glass-ceramic nanoparticles (A) and containing 25 wt% bioactive glass-ceramic nanoparticles (B).

Figure 11. SEM micrographs of fracture surfaces of PLLA/bioactive glass (BG) nanocomposites developed by Liu et al. [91]: 4 wt.% surface modified-BG (A), 4 wt.% BG (B), 20 wt.% surface modified -BG (C) and 20 wt.% BG (D) (Reproduced from ref. [91] with permission of Elsevier).

Figure 12. SEM morphology of a BGNF-Col nanocomposite, formulated as (a,b) thin membrane and (c,d) porous scaffold. Parts of (a) and (c) are enlarged in (b) and (d), respectively [100]. (Reproduced from ref. [100] with permission of John Wiley and Sons)

Figure 13. Elastic modulus of PCL control, PCL/20 wt % BGP composite and PCL/20 wt % BGNF composite. (n=5, *p<0.05, **p<0.01) investigated by Jo et al. [102]. (Reproduced from ref. [102] with permission of John Wiley and Sons)

FIGURE 1

FIGURE 2

FIGURE 3

(A)

(B)

FIGURE 4

FIGURE 5

(a)

(b)

Figure 6

FIGURE 7

Figure 8

Figure 9

Figure 10

Figure 11

Figure 12

Figure 13