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Glasses in bone regeneration: A multiscale issue

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ABSTRACT

3D scaffolds based in mesoporous bioactive glasses (MBGs) are being widely investigated to use in bone tissue engineering (TE) applications. These scaffolds are often obtained by rapid prototyping (RP) and exhibit an array of interconnected pores in a hierarchy of sizes. The ordered mesopore network (around 4 nm in diameter) is optimal for the adsorption and release of bone inductor biomolecules, and the arrangement of macropores over 100 μm facilitates the bone cell ingrowths and angiogenesis. Nevertheless MBG composition can be varied almost infinitely at the atomic scale by including in the glass network oxides of inorganic elements with a therapeutic action. In this article the synthesis and characterization of MBG scaffolds based on the 80%SiO_2–15%CaO–5%P_2O_5 (in mol-%) glass with substitutions up to 3.5% of Ga_2O_3 or Ce_2O_3 or 7.0% of ZnO are revisited. The substituent inclusion and the RP processing slightly decrease the surface area, the pore volume and the mesoporous order as well as their bioactive response in solutions mimicking blood plasma. However, these values still remain useful for bone TE applications. Results exhibiting the bactericide action of MBG scaffolds containing ZnO are also presented.

1. Introduction

Recently bioactive glasses have been widely investigated for diverse clinical applications including the tissue engineering (TE) of bone. Several families can be distinguished in the historical evolution of the bioactive glass investigation, beginning with the first Melt Prepared Glasses (MPGs), discovered by Hench et al. in 1969 [1], followed by the bioactive Sol–gel Glasses (SGGs) also proposed by Hench in 1991 [2] and the most recent, glasses with ordered mesoporosity designed by Zhao and Vallet-Regi, who investigated independently and reported them for the first time in 2004 and 2006 [3,4]. These glasses denoted as Mesoporous Bioactive Glasses (MBGs) are the main subject of this article.

Simultaneous to the development of glasses with characteristics more and more adapted for use as implant materials, there was a change in the paradigm of the role of bioactive materials used in Orthopedics and Dentistry. In the past, there was a search for materials for the simple substitution and repair of osseous tissues. In 2002 Hench and Polack proposed the search of materials aimed to drive and favor the regeneration of bone, denoted as third generation biomaterials [5]. In their studies they demonstrated that the silicon and calcium ions released from bioactive melt glasses as granules stimulated the genes to persuade the cells to form bone.

The so-called third generation biomaterials can be directly classified in the field of the TE of bone. The first generation are as bioinert as possible and the bioactive or resorbable biomaterials are considered the second generation. It is well known that TE is based on three fundamental pillars: cells, signal molecules and scaffolds [6]. Is in this last pillar, where the MBGs processed into 3D scaffolds are considered as an optimal option. An important requirement is that these scaffolds exhibit an interconnected and hierarchical porosity, that is to say, with several orders of magnitude including giant pores (channels) and macropores that allow internal angiogenesis and the interaction with cells. Nevertheless, pores of nanometric size are also required like those exhibited by the mesoporous materials. Such pores allow the inclusion of signal molecules that induce the formation of bone like the Bone Morphogenetic Proteins (BMPs), growth factors like Vascular Endothelial Growth Factor (VEGF), or different fractions of Parathyroid Hormone related Peptide (PTHrP) [7].

With all these ideas in mind, in the last few years CaO–P_2O_5–SiO_2 based MBG scaffolds (MBG_Sc) have been widely investigated as optimum candidates for bone TE applications. Effectively all these features have special characteristics for the role they must play when implanted for the regeneration of osseous tissues. First, the ordered mesoporosity allows the adsorption and release in a controlled manner of the bone induction agents previously mentioned. In addition, these mesoporous channels confer extremely high values of surface area and pore volume to MBGs which increase the already high levels of bioactivity of CaO–P_2O_5–SiO_2 glasses obtained by the quenching of a melt or by sol–gel processing [8,9]. Keeping in mind the essential properties of a 3D scaffold

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for bone TE applications, several strategies have been proposed for the design of a macroporosity required for cell functions such as the bone cell ingrowths, the nutrient supply and vascularization, as well as for the adhesion and development of the bone cells. These strategies include foaming, freeze drying, fiber bonding or rapid prototyping (RP) technologies [10]. Obviously, in all cases it is very important to confirm that the MBG powder processing to obtain the scaffolds does not destroy the ordered mesoporosity and bioactivity.

On the other hand, because MBGs are glasses they do not exhibit an exact composition and can be substituted with small amounts of extra oxides of cations featuring important biological actions, such as osteogenesis, antibacterial capacity, angiogenesis or cementogenesis [11–13]. For this reason, there is a general trend to upgrade properties with some of the well known therapeutical ions because of their biological action [14–16]. Fig. 1 depicts an approximate time required for in vitro bioactivity for the three families of bioactive glasses and the year of discovering of each family. The biological action of inorganic ions used for upgrading the glasses is also included.

The three families of bioactive glasses showed in Fig. 1 are silicate-based glasses, with the presence of CaO as essential requirement for bioactivity. The third component used to be P_2O_5 which binds calcium producing calcium phosphate nuclei that completely modifies the reactivity of glasses when in contact with biological fluids [17]. The development of the three families of glasses meant an increase of the textural properties (surface area and pore volume) in going from the dense MPGs to MBGs exhibiting more than 500 m^2/g of the surface area and 0.5 cm^3/g of the pore volume. The surface area and porosity of MBGs are practically half of pure silica mesoporous materials such as MCM-41 or SBA-15, but they are more than twofold the values of sol–gel glasses with analogous composition [18]. Noticeably such increase of textural properties produces an increase in the kinetics of the bioactive response. Such increase is relatively small in terms of the bone formation induced by these glasses. However, more important is the new capabilities in terms of the ability to include and release biologically active substances in the MBG pores.

Regarding the biological effect of the extra inorganic ions included in the glasses, *osteogenesis* was the first feature investigated for bone TE applications. For this reason one of the most important property searched for the extra elements added to upgrade the SiO_2–CaO–P_2O_5 glasses was its *osteogenic* character. In addition, one of the main problems when a material is implanted in bone is osteomyelitis caused for bacterial infection. Thus, a second important property investigated was the ability of fight against infection. For this reason elements with *antibacterial* ability have been also investigated. Because of an application of bioactive glasses as grafts in Dentistry, inorganic ions with *cementogenic* properties were also investigated. Finally to fulfill some of the mentioned properties is necessary for the formation of blood vessels. Consequently the addition of *angiogenic* elements is also of interest.

The main objective of this paper was to review the state of the art and perspectives of future of MBG scaffolds based on the 15%CaO–5%P_2O_5–80%SiO_2 glass enriched with extra cations to add extra features when implanted that made them more valuable when implanted for the bone tissue regeneration. Specifically our research group was very interested in the last years in the substitution of MBGs by: (i) up to 3.5% Ce_2O_3, because it was reported that Ce^{3+} ions reduce the enamel demineralization, and are neuroprotective [19], (ii) up to 3.5% Ga_2O_3, because it was published that Ga^{3+} ions increase the calcium content in bone, and are antimicrobial [20], and (iii) ZnO because, in addition to other biological functions, it was reported that Zn^{2+} ions stimulate the bone formation and exhibit also a bactericide action [21,22]. In Fig. 2 the role of the features of the MBG_Sc all of them playing an essential role in bone TE are depicted. The biological actions of Ce^{3+}, Ga^{3+} and Zn^{2+} ions, that will be deeply reviewed in this paper, are highlighted in the center of the Figure.

2. Mesoporosity, textural properties and bioactivity of substituted MBG powders

SiO_2–CaO–P_2O_5–X (X = Ce_2O_3, Ga_2O_3 or ZnO) MBG powders are synthesized by the evaporation induced self-assembly (EISA) method [23]. As reactants are generally used tetraethyl orthosilicate, triethyl phosphate, calcium nitrate tetrahydrate and cerium, gallium or zinc nitrates are dissolved in ethanol and adding nitric acid as catalyst and Pluronic® P123 as surfactant [24]. The sol obtained is poured in a Petri dish for the solvent evaporation. Resultant materials are thermally treated for the surfactant and nitrate removal to obtain MBG powders. Other approaches for the synthesis of MBGs can be found in Ref. [25].

Fig. 3 shows some results obtained after characterization of MBG *powders* with compositions obtained by substituting the 15%CaO–5%P_2O_5–80%SiO_2 (in mol-%) glass with up to 3.5% Ce_2O_3 or Ga_2O_3 or up to 7.0% ZnO. In Fig. 3a maxima in the Low Angle X-ray Diffraction (LA-XRD) region can be observed, indicative of mesoporous ordered arrangements. MBGs lack long distance structural order because they are glasses and do not exhibit diffraction maxima in the Wide Angle XRD patterns (WA-XRD). If we observe the LA-XRD pattern unsubstituted glass (thereafter will be denoted as blank, B), a sharp maximum at 1.4° in 2θ, that can be indexed to the (10) reflection of a

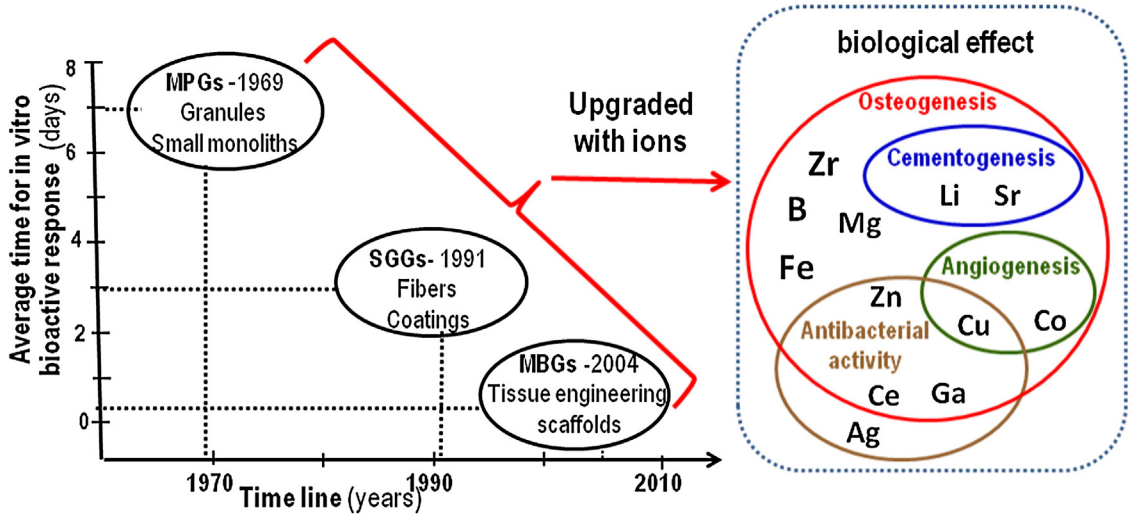

Fig. 1. Left: timeline in the discovering of the three families of bioactive glasses and the approximate time required for a standard member of each family was coated by an apatite-like layer after being soaked in a simulated body fluid. Right: some ions investigated to upgrade the glasses and their biological effects.

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