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Designing antimicrobial bioactive glass materials with embedded metal ions synthesized by the sol–gel method

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ABSTRACT

Bioactive glasses (SiO_2–P_2O_5–CaO) having tailored concentrations of different biocide metal ions (copper or silver) were produced by the sol–gel method. All the particles release phosphorous ions when immersed in water and simulated body fluid (SBF). Moreover, a surface layer of polycrystalline hydroxy-carbonate apatite was formed on the particle surfaces after 10 day immersion in SBF as confirmed by X-ray diffraction and scanning electron microscopy (SEM) showing the bioactive materials. Samples with embedded either copper or silver ions were able to further release the biocide ions with a release rate that depends on the metal embedded and the dissolution medium: water or SBF. This biocide ion release from the samples explains the antimicrobial effect of our active particles against *Escherichia coli* DH5α ampicillin-resistant (Gram-negative) and *Streptococcus mutans* (Gram-positive) as determined by the Minimum Bactericidal Concentration (MBC) method. The antimicrobial behavior of the particles depends on the bacteria and the biocide ion used. Noteworthy, although samples with copper are able to release more metal ion than samples with silver, they present higher MBC showing the high effect of silver against these bacteria.

1. Introduction

Biomaterials have become a relevant part of modern medical sciences in such areas related with trauma or restoration of human body functions. Examples of application of these materials are: hard tissue dental implants, load-bearing hip prostheses, bone screws, alveolar ridge (jaw bone) and maxillofacial reconstruction, ossicular (middle ear) bone substitutes, keratoprostheses (corneal replacements), segmental bone replacements, implants in middle-ear surgery to replace ossicles, percutaneous devices, alveolar ridge augmentation, otolaryngology, prosthetic heart valves, orbital floor repair, tissue engineering, and bone regeneration in general, among others [1–4]. However, these materials present some limitations related with the occurrence of infections. These so-called biomaterials centered infections (BCI) are responsible of several problems from the non-optimum implant device performance to lethal sepsis of the patient. Implant mobilization and failure are often determined by these infections that, once become chronic, do not respond any longer to conventional antibiotic therapy with considerable costs and suffers to the patient. The cost to treat BCI is tremendous, estimated to be at least $50,000 per patient having as consequence a cost of $250 million per year only in the USA [5]. The incidence of BCI and its consequences varies depending on the specific uses of the biomaterial [6–10]. In this way, implant infection, besides being the principal cause of implant failure and an unresolved problem to the clinicians, is still an open scientific challenge [11].

Microorganisms can reach the biomaterial before and during its implantation but also after when hematogenous infections caused by a bacteremia are produced. The latter is generally originated by skin infections, surgical or dental interventions, pneumonia, abscesses, or bacteriuria [8,12]. The complexity of BCI is further related with the formation of a biofilm (bacteria encased in a self-produced matrix) produced by the microorganism having more resistant to antibiotics than individual colonies [13,14]. Noteworthy, a deep implant infection can show clinical signs after a year of the microbial seeding as the biofilm formed can stay silent for long periods [8]. In this way, the approach based on the prevention of the biofilm formation by reducing the growth of microorganisms adhered on the surface seems to be a plausible method avoiding BCI [14].

Different kinds of biomaterials are routinely employed and among them bioactive glasses are widely used in the fields of dentistry and orthopedics as they pushed the boundaries of biomaterial capability and function. These ceramics changed the paradigm of biomaterials 40 years ago from bioinert to bioactive materials showing a strong active response, such as osteoproductivity, after they are implanted in the human body. Moreover, these glasses available through bioerodible gel systems have shown significant remineralization properties with the potential to be a major advancement in, for example, the clinical

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management of early caries lesions [15]. A series of chemical transformations occurs after implantation of a bioactive glass leading to the growth of a layer of crystalline hydroxy-carbonate apatite (HCA) on its surface explaining its bioactivity [16,17]. The reaction steps or stages leading to the HCA formation have been roughly identified: (a) initial leaching of Na^+ ions; (b) breaking of Si – O – Si bonds and releasing of soluble silica to the solution; (c) formation of a silica-rich layer on the surface; (d) formation of an amorphous calcium phosphate phase on the surface; and (e) crystallization of the last amorphous film into HCA. Recent studies however show that the role of the bioactive glasses is primarily to release the critical concentration of biologically active ions at the rate needed for cell proliferation and differentiation [18]. All early bioactive glass ceramic processing involved melting the glass phase at high temperature followed by casting or quenching of powders. However, a stable bioactive glass could also be produced by sol–gel method [19]. The pore structure formed during this process increases the specific surface area by two orders of magnitude compared to a melt-derived glass of a similar composition. Noteworthy, the rate of surface HCA formation for this sol–gel based material was more rapid than the melt derived. Sol–gel method allows the production of a two (CaO and SiO_2), three (SiO_2–CaO–P_2O_5) or even four (SiO_2–CaO–P_2O_5–Ag_2O) component bioactive glass [18–21]. Other methods are further reported focusing on synthesis of bioactive glass materials with improved textural properties such as ordered mesoporous materials having even larger specific surface area than sol–gel based glasses [22]. The impact of bioactive glass materials can be confirmed by the large field of applications where they are involved such as: coatings for orthopedic metallic implants, trabecular coatings, bone replacement, periodontology, endodontology, scaffolds for bone tissue engineering, regenerative medicine, and composite based scaffolds, among others [23–27].

Based on the above mentioned, it is reasonable to postulate that the ideal bioactive glass material would be one with antibacterial elements preventing infections and thus reducing the post-operative care and the recovery time of a patient [28]. In this context, metals are widely considered because of its bioactivity against microorganisms overcoming also the problems related with the low stability of other organic antimicrobial compounds during the biomaterial processing. Silver ions have been one the most studied metals as it presents a broad and strong antibacterial behavior [28]. Silver has been introduced into silicate and phosphate based glass-ceramics during the sol–gel [29–34] and the molten salt ion exchange [28,35–37] techniques. Williams et al. showed that a bioactive glass doped with Ag_2O presents bacteriostatic and bactericidal properties against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [33]. The same authors showed that the antibacterial action of these novel materials is attributed exclusively to the leaching of Ag^+ ions from the glass matrix [33] as later confirmed in another sol–gel bioactive glass system [22]. Moreover, bioactive glass scaffolds containing silver released its ions at a rate enough to be bactericidal but not high enough to be cytotoxic to bone cells [19].These novel antimicrobial bioactive systems can be used as filler for other biomaterials [38]. Despite these results focus on silver, copper can also be used in antimicrobial applications with even improved performance from the biomaterial point of view. The antimicrobial behavior of copper and its alloys, such as brasses, bronzes, copper-nickel and copper-nickel-zinc, among others, has been extensively demonstrated during the last years against different bacteria associated to transmission *via* [39]. The strong antimicrobial effect found can be rendered to different matrices such as those based on polymers or ceramics [40–43]. The advantages of copper are not only related with its wide range of antimicrobial effectivity, but also with its low toxicity to humans [39]. Noteworthy, copper is much more than only an excellent antimicrobial agent having other relevant bioactivities. Similar to many other micronutrients, it has an essential role in bone formation and healing [33]. Inorganic copper exerts wound healing responses *in vivo* and *in vitro* and improves the vascular density in and around subcutaneously implanted allografts and hyaluronan based hydrogel [44,45]. Copper sulfate is a perfect additive for blood vessel ingrowth inducing the formation of cord-like and tubular structures and potentiating the effect of endogenous growth factors [44,46]. Remarkable distributions of cellular copper have been found in human endothelial cells when they were induced to undergo angiogenesis revealing the importance of this ion as an angiogenic agent [47,48]. Another study revealed that copper, associated with angiogenesis growth factor FGF-2, promotes synergetic stimulating effects on angiogenesis *in vitro* [46]. Moreover, this ion can stimulate the proliferation of human endothelial cells [49] and inhibit osteoclast activity [50]. By releasing copper ions from nanoparticles, an aggregation of elastin fibrils into mature fibers was further found suggesting that elastin matrix deposition is stimulated by this metal with enhanced crosslinking [51]. Despite all the above mentioned properties, the synthesis of sol–gel based bioactive glass materials with embedded copper ions has been rarely published [43].

The goal of the present article is to synthesize sol–gel based bioactive glasses with different amount of copper ions focusing on its antimicrobial effect as compared with the same material doped with silver ions. Our results show that the incorporation of biocide metal ions inside the bioactive material does not alter the formation of hydroxyapatite on its surface after immersion in simulated body fluid (SBF). Moreover, glasses containing biocide metal ions present strong antimicrobial behavior depending on the metal used and the specific bacteria tested.

2. Experimental

2.1. Synthesis

The sol–gel based SiO_2–P_2O_5–CaO bioactive glass materials were synthesized from tetraethoxysilane (TEOS; Aldrich) based on previously reported information [21]. The pure bioactive material (BG) consists in a standard bioactive glass without biocide metal ions with a composition of 62.3 wt.% SiO_2, 28.9 wt.% CaO, and 8.6 wt.% P_2O_5 (Table 1). Initially, TEOS was added into 0.1 M of nitric acid and mixed for 60 min at room temperature. Afterward, triethylphosphate (TEP; Aldrich) was added and elapsed 45 min calcium nitrate tetrahydrate (Aldrich) was further added, and the system was finally stirred for 60 min finishing the hydrolysis reactions. For the bioactive glasses with copper ions (CuBG), after the addition of calcium nitrate tetrahydrate a certain amount of copper acetate is added and the system is stirred for 1 h controlling the pH (1.4) by adding nitric acid. For the bioactive glasses with

Table 1
Main characteristics of the different sol–gel based bioactive glass materials.

Sample	Average diameter [μm]	SSA* [m^2/g]	SiO_2** [wt.%]	CaO** [wt.%]	P_2O_5** [wt.%]	CuO**,*** [wt.%]	Ag_2O**,*** [wt.%]
BG	114.6	161.1	62.6/70.3	28.9/19.4	8.6/10.3	–	–
CuBG1	103.9	–	62.3/73.5	28.7/17.3	4.3/5.5	4.7/3.7	–
CuBG2	124.9	54.7	58.0/67.7	29.0/18.9	4.0/5.0	9.0/8.4	–
AgBG1	95.2	–	62.8/72.0	28.9/17.4	4.3/5.3	–	4.0/5.3
AgBG2	82.9	32.3	58.0/70.1	29.0/15.2	4.0/4.7	–	8.0/10.0

*Specific surface area; **theoretical (left) and experimental (right) values; ***reference values assuming 100% metal oxide compounds.