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Magnesium calcium silicate bioactive glass doped with copper ions; synthesis and *in-vitro* bioactivity characterization

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ABSTRACT

Recent studies revealed that metallic ions have therapeutic effects on human bone metabolism and angiogenesis. Thus, in this study, a new magnesium-calcium-silicate glass powder, being doped with copper ions was synthesized through the sol-gel process; the material was termed as 60S7M2Cu. The glass was thermally treated at several temperatures up to 870 °C in order to induce crystallization, which would potentially enhance its mechanical and/or biological properties. Structural and morphological characterization, thermal analysis and *in-vitro* apatite forming ability evaluation were performed by X-ray diffraction-XRD, Scanning Electron Microscopy-SEM coupled with Energy Dispersion Spectrometry-EDS, Thermogravimetric Analysis, as well as Fourier Transformed Infrared-FTIR Spectrometry. Moreover, the elemental release was recorded by Inductively Coupled Plasma Atomic Emission Spectroscopy ICP-AES. Experimental results showed that a crystalline hydroxyapatite phase was developed after 3 days in the simulated body fluid (SBF) followed, after 15 days, by the growth of a carbon hydroxyapatite layer, in both untreated glass as well as treated glass-ceramics nearly at all temperatures. At lower temperatures, namely 770 and 800 °C, a whitlockite bioactive phase appeared after the immersion in SBF, while a wollastonite crystalline phase emerged when treated at 830 and 870 °C without having a negative effect on its apatite forming ability. Copper ions releasing from the glass and glass-ceramics materials was proved to be slow and stable during the whole period of soaking. In this study copper containing glass and glass-ceramics, which present apatite forming ability and according to literature possess the capability to induce the release of therapeutic ions with osteogenic and angiogenic capacity, were synthesized. Eventually, after further investigations, this glass composition could be applied in bone tissue engineering, where bioactive materials able to induce simultaneously both osteogenesis and angiogenesis constitute a constant demand.

1. Introduction

Bioactive fixation [1] is a term adopted for a series of biophysical and biochemical reactions that eventually result in an interfacial bonding of an implant to the tissue. All the *in-vitro* and *in-vivo* tests results show that among others, the only common characteristic of the known bioactive implant materials is the formation of a biologically active hydroxyapatite (HCAp) ($Ca_5(PO_4)_3(OH)$) layer on the implant surface [1–4]. There are many advantages of bioactive fixation, among which the most straightforward is dealing with the strength of a bioactive bond at the corresponding implant-bone interface [1]. For several years now, inorganic bioactive materials have been used for bone tissue regeneration. The reaction of an amorphous silica-based material with the human physiological serum, resulting in the formation of a crystalline hydroxyapatite layer, stands as the cornerstone of bioactive ability. Nevertheless, the elapsed time before the formation of this layer stands as the most stringent marker for bioactive response. The only, unique requirement for this specific biological response is the presence of the human body physiological environment.

The development of biomaterials with novel properties has always been a stimulating motivation upon research. Until the late 1980s, the main bioactive materials used were generally melt-derived, with the majority of research aiming at the bioactive glasses in the SiO_2-Na_2O-CaO-P_2O_5 system [1]. Nevertheless, apatite-wollastonite (the short form A/W being most commonly used) bioactive glass-ceramics containing crystalline oxyfluoroapatite [$Ca_{10}(PO_4)_6(O,F)_2$] [5] were also the

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subject of intense research. The first bioactive glass manufactured ever was the 45S5 Bioglass® [2], also known as the Hench bioglass, which is a melt derived, quaternary glass-ceramic with composition (in wt%): 45 SiO_2 – 24.5 Na_2O - 24.5 CaO - 6 P_2O_5 [6–10]. A rapid rate of HCAp formation [7] has been observed for the cases of melt-derived bioactive glasses, which was mostly attributed to the presence of Na_2O or other alkali cations in the glass composition. A continuous effort in the synthesis of new and improved biomaterials has been occurred, by seeking more and more the enhancement of performance. Towards that direction, various glass synthesis routes such as sol-gel or hydrothermal methods have been selected as more efficient compared to conventional melt-quenching [11], while the incorporation of ions such as Ag, Cu, Mg, Zn etc., has led to glass compositions with advanced antibacterial or anti-inflammatory properties [12]. Apart from being able to induce apatite formation, their ionic products were shown to enhance the proliferation, osteogenic differentiation and gene expression of stem cells [13–15] and their angiogenic and osteogenic capacity [16, 17], thus improving their overall tissue regeneration efficiency [12, 18]. Since biomaterials are quite complex multi-component substances, improving their chemical, physical and biological properties still stands as an interesting research area. Therefore, glass systems were created by modifying their formula (and hence their structure) as well as by moving to glass systems with varying components, such as binary and ternary bioglasses and even bioglasses with much more complex formulations [12].

By the route of sol–gel method, a colloidal solution (sol) gradually evolves through polycondensation reactions, towards the formation of liquid starting compounds, to a gel, and finally to a dry network that is applied towards the preparation of advanced glasses and ceramics [19]. The main features of the multi-component sol–gel process are the following: a) the formation of a homogeneous solution before polymerization, b) the relatively low processing temperatures and c) the ease of powder production [20], resulting thus, not only in chemical homogeneity, but also in phase separation, crystallization and lack of chemical de-composition [21]. Unlike to the melt-derived method, sol-gel derived powders exhibit intrinsically higher surface areas and a high level of porosity in the mesoporous range. This fact promotes a higher degree of hydroxylation of the surface forming a silica-rich gel layer, which plays a significant role in HCAp nucleation. [22–25]. Furthermore, because of their mesoporous texture and high surface area, sol-gel derived materials can absorb a range of substances such as proteins and cells. Therefore they become an alternative for preparing glasses for a number of applications in the biomedical field [26].

A third generation of biomaterials has been described by Hench and Polack [27]; these scientists tried to combine the features of biocompatibility and biodegradability towards enhancing the growth of specific cells. The result was a new generation of more functional biomaterials, such as Mg-based materials [28]. Magnesium has been reported to (a) play a crucial role in human bone growth, maintenance and repair by stimulating osteoblast proliferation [29] and (b) be involved in over three hundred chemical reactions in the human body, with the most important among these being the regulation of the transport of calcium [30, 31]. Moreover, magnesium and calcium oxides are behaving very much likely within a silicate glass structure, as both act as network modifiers [32]; thus a trend has been identified, progressively replacing CaO by MgO [33, 34]. MgO may, alternatively, act as intermediate oxide in highly disrupted silicate glasses from the extension of the glass forming region in ternary diagrams towards lower SiO_2 mole fractions [29, 31, 32] and references therein; its function in bioactive glass formulations has been the subject of various conflicting explanations and discussions [30].

Recently, copper, strontium, zinc, cobalt, silver and boron, have been used as potential therapeutic agents due to their ability to enhance bone formation due to their stimulating effects on both osteogenesis and angiogenesis [35–37]; these ions are termed as “bioinorganics”. Some of them (e.g. copper, zinc, silver) exhibit additional therapeutic effects, like anti-inflammatory and antibiotic capabilities [38, 39]. Thus, the knowledge of the effects of inorganic ions on health has focused the research interest on the addition of inorganic ions into biomaterials. With the only precaution that these ions are always in strictly indicated quantities, this innovation is reported to be beneficial to humans, while inorganic ions are quite efficient in accelerating the healing of bone tissue disease [35, 40]. Specifically speaking, copper ions are believed to participate in blood vessel formation, known as angiogenesis [41] and also to have a catalytic contribution in hematopoietic stem cell (HSC) culture [42].

Looking into the future, the development of new types of multifunctional bioactive glasses that merge their intrinsic bioactive behavior with therapeutic inorganic ions carrier ability [43, 44] could be feasible. In the framework of the requirement for (a) novel materials with controlled degradability, (b) well-defined and predictable ion release kinetics and (c) specific surface area and porosity of the scaffolds [37], a new synthesis scenario was adopted in this study, integrating different concepts such as the application of the sol-gel technique, the use of both magnesium oxide and calcium oxide as network modifiers and the addition of copper ions that stimulates angiogenesis and promotes formation (and maturation) of blood vessels. Consequently, the aim of the present work was the synthesis, characterization and the *in-vitro* apatite forming ability of a new quaternary glass-ceramic in the system SiO_2-CaO-MgO-CuO with 2.5 in wt% of CuO, hereafter 60S7M2Cu. Finally, various heating treatments were applied in order to determine an appropriate nucleation-crystallization temperature, where the apatite forming ability of the glass is not affected.

2. Materials and experimental methods

2.1. Reagents

Chemicals in this work were used as obtained: Tetraethylorthosilicate (TEOS), 2N HNO_3, $Ca(NO_3)_2{\cdot}4H_2O$, $Mg(NO_3)_2{\cdot}6H_2O$, $Cu(NO_3)_2{\cdot}2.5H_2O$ as starting materials for preparation of bioactive glass and reagent-grade chemicals NaCl, $NaHCO_3$, KCl, $K_2HPO_4{\cdot}3H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2$, Na_2SO_4 trishydroxymethyl aminomethane [Tris-buffer, $(CH_2OH)_3CNH_2$], and 1N HCl are the required materials for preparation of SBF. All reagents were purchased form Sigma-Aldrich (ACS reagents, 98% purity or higher).

2.2. Synthesis of magnesium calcium silicate glass

The glass (SiO_2 60, CaO 30, MgO 7.5, CuO 2.5 in wt%) was prepared by the sol-gel method as described in literature [45]. More specifically, 20 ml TEOS, 19.4 ml distilled H_2O and 3.2 ml (2N) HNO_3 were mixed and they were stirred until the solution became clear. Subsequently, 10.576 g $Ca(NO_3)_2{\cdot}4H_2O$, 2.871 g $Mg(NO_3)_2{\cdot}6H_2O$ and 0.868 g $Cu(NO_3)_2{\cdot}2.5H_2O$ were added while the solution was constantly stirring in room temperature. After mixing all the components, the solution was transferred in a furnace at 60 °C for 2 days and 7 h to dry and produce a xerogel. Afterwards the xerogel was moulded in a Pt crucible that was inserted into a high temperature furnace, in order to be sintered gradually up to 130 °C for 2 days and 21 h and right after it was stabilized at 700 °C for 3 h. Part of the product was grinded and sieved into powder of < 40 μm, hereafter called as the untreated material.

2.3. Preparation of SBF

The SBF solution was prepared by dissolving reagent-grade NaCl, KCl, $NaHCO_3$, $MgCl_2{\cdot}6H_2O$, $CaCl_2$, Na_2SO_4 and $K_2HPO_4{\cdot}3H_2O$ into distilled water and buffered at pH = 7.25 with TRIS (trishydroxymethyl aminomethane) and HCl 1 N at 37 °C according to Kokubo et al. [46]. Its ion composition is given in Table 1 and is compared with the human blood plasma.