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Prevention of bacterial adhesion to zwitterionic biocompatible mesoporous glasses

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ABSTRACT

Novel materials, based on Mesoporous Bioactive Glasses (MBGs) in the ternary system SiO_2-CaO-P_2O_5, decorated with (3-aminopropyl)triethoxysilane (APTES) and subsequently with amino acid Lysine (Lys), by post-grafting method on the external surface of the glasses (named MBG-NH_2 and MBG-Lys), are reported. The surface functionalization with organic groups did not damage the mesoporous network and their structural and textural properties were also preserved despite the high solubility of MBG matrices. The incorporation of Lys confers a zwitterionic nature to these MBG materials due to the presence of adjacent amine and carboxylic groups in the external surface. At physiologic pH, this coexistence of basic amine and carboxilic acid groups from anchored Lys provided zero surface charge named zwitterionic effect. This behaviour could give rise to potential applications of antibacterial adhesion. Therefore, in order to assess the influence of zwitterionic nature in *in vitro* bacterial adhesion, studies were carried out with *Staphylococcus aureus*. It was demonstrated that the efficient interaction of these zwitterionic pairs onto the MBG surfaces reduced bacterial adhesion up to 99.9% compared to bare MBGs. In order to test the suitability of zwitterionic MBGs materials as bone grafts, their cytocompatibility was investigated *in vitro* with MC3T3-E1 preosteoblasts. These findings suggested that the proposed surface functionalization strategy provided MBG materials with notable antibacterial adhesion properties, hence making these materials promising candidates for local bone infection therapy.

Statement of Significance

The present research work is focused in finding a preventive treatment of bone infection based on Mesoporous Bioactive Glasses (MBGs) with antibacterial adhesion properties obtained by zwitterionic surface modification. MBGs exhibit unique nanostructural, textural and bioactive characteristics. The novelty and originality of this manuscript is based on the design and optimization of a straightforward functionalization method capable of providing MBGs with zwitterionic surfaces that are able to inhibit bacterial adhesion without affecting their cytocompatibility. This new characteristic enhanced the MBG properties to avoid the bacterial adherence onto the implant surfaces for bone tissue engineering applications. Subsequently, it could help to decrease the infection rates after implantation surgery, which represents one of the most serious complications associated to surgical treatments of bone diseases and fractures.

1. Introduction

The so called third generation biomaterials can be directly classified in the field of bone tissue engineering. Since 2004, when Chen et al. prepared for the first time highly ordered Mesoporous Bioactive Glasses (MBGs) with superior *in vitro* bone-forming bioactivities [1], MBGs based in the ternary system SiO_2-CaO-P_2O_5 have been widely investigated and regarded as optimum candidates for lost bone regeneration. Moreover, the ordered mesoporous arrangement, with high surfaces and pore volume, allows using MBGs in drug delivery systems for the treatment of bone tissue diseases [2,3]. In addition, the high amount of free silanol groups present on MBG surfaces allows to open an interesting

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route to the anchoring of new species through a covalent attachment, thus improving the applications of these materials [4,5].

Post-operative implant infections are one of the most serious complications associated with surgical treatments of bone diseases and fractures with bone grafts and prostheses [6,7]. In the issue of osteomyelitis (an inflammatory process that leads to bone destruction), the bacteria of biggest concern include *Staphylococcus aureus* and *Staphylococcus epidermidis* [8,9]. Bacteria typically secrete polymeric materials after their association to form protective coatings known as biofilms. Biofilms have been defined as "aggregates of microorganisms in which cells are frequently embedded in a self-produced matrix of extracellular polymeric substances that are adherent to each other and/or a surface" [10]. The biofilm further impedes the activity of the host defenses and/or antibiotic therapy, requiring surgical intervention to remove the implant as the only effective option. According to the National Healthcare Safety Network (NHSN) between 2006 and 2008, the postoperative infection rates associated with orthopaedic surgery range from 1.11 to 3.36% for open reduction of fracture, from 0.58 to 1.60% for knee replacement and from 0.67 to 2.40% for hip replacement, depending on patient risk. Applying these rates to the total amount of hip and knee replacements performed in the United States results in an estimated 6000–20,000 events of surgical site infection per year, associated with hip and knee replacements alone [11,12]. More recently, NHSN reported a 44.2% of surgical site infections by *S. aureus* in open reduction of fracture, hip prosthesis and knee prosthesis among others between 2011 and 2014 [13]. This problem brings serious financial consequences for both, patients and healthcare provider, because the most effective treatment is the implant removal by surgical intervention, with the subsequent cost increase [14].

Zwitterionic polymers, such as poly(carboxybetaine methacrylate) and poly(sulfobetaine methacrylate), containing quaternary ammonium as a positive charge and carboxylate and sulfate as negative charges, have been reported to be good ultralow fouling materials [15]. These zwitterionic-based materials are receiving great attention due to their effectiveness, robustness, and stability [16,17]. Recently, zwitterionization of biomaterials has emerged as a groundbreaking strategy to endow surfaces with high resistance against non-specific protein adsorption, bacterial adhesion and/or biofilm formation for dentistry or orthopaedics applications [18,19]. Vallet-Regí research group have reported a straightforward way to synthesize mesoporous silica ceramics exhibiting zwitterionic surfaces that are able to inhibit bacterial adhesion, whereas allowing osteoblast adhesion and proliferation under *in vitro* conditions [7,20–22]. In this work, a novel scientific effort is devoted to provide bioactive bioceramics such as MBGs with antibacterial adhesion capability while preserving their biocompatible behaviour.

Zwitterionic species are characterized by having an equal number of positively and negatively charged groups within a molecule, hence maintaining overall electrical neutrality. It can bind water molecules even more strongly than hydrophilic materials via electrostatically induced hydration, becoming an important part in achieving interfacial bioadhesion resistance [23,24]. Here we present MBGs with zwitterionic functionalization through amino acid incorporation to the surface. Amino acids are well-known natural zwitterions compounds, having a carboxyl group (–COOH) and an amine group ($–NH_2$) with a third reactive group linkable with MBG surfaces. Several authors have reported the usefulness of amino acid as low fouling strategies to avoid bacterial adhesion [15,25].

Herein, we report for the first time the design and synthesis of a new nanostructured zwitterionic MBGs with antibiofouling capability that inhibits bacterial adhesion. The cytocompatibility of non-zwitterionic and zwitterionic MBG materials was investigated *in vitro* with MC3T3-E1 preosteoblasts cells.

2. Materials and methods

All chemicals were purchased from Sigma-Aldrich, except (3-aminopropyl)triethoxysilane (APTES, 97% wt) that was purchased by ABCR, and used as received without further purification. All surface functionalization reactions were carried out under an inert atmosphere using Schlenk techniques and anhydride solvents.

2.1. 75/85-MBGs synthesis

Ordered mesoporous materials in the ternary SiO_2-CaO-P_2O_5 system were obtained as described in Ref. [26]. Briefly, the MBGs were synthesized by using evaporation induced self-assembly (EISA) [27] method and non-ionic surfactant $EO_{20}PO_{70}EO_{20}$ (Pluronic P123) as structure directing agent. Tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP), and calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) were used as SiO_2, P_2O_5 and CaO sources, respectively. Two different compositions were synthesized, denoted as 85-MBG and 75-MBG. The amount of reactants and nominal compositions are collected in Table 1. The resulting transparent membranes were calcined at 700 °C in air for 6 hours to obtain the finally 75-MBG and 85-MBG powders. Finally, the powders were sieved below the 63 μm. The final compositions of 75-MBG and 85-MBG samples were calculated by X-ray fluorescence (XRF) (Table 1).

2.2. 75/85-MBG surface functionalization with (3-aminopropyl) triethoxysilane (APTES)

75-MBG and 85-MBG samples were surface functionalized directly post-synthesis with amine groups using (3-aminopropyl) triethoxysilane (APTES). A sample of 2 g of 75/85-MBG was dried and degassed overnight at 110 °C. Afterward, 75/85-MBG was dispersed in 20 mL anhydrous toluene and the suspension was stabilized at N2 atmosphere. Subsequently, a colorless solution formed by 7.2 mL (30.8 mmol) APTES and 25 mL dry toluene was added dropwise, with constant stirring, to a suspension of 75/85-MBG. The mixture was refluxed 24 h at 80 °C under N2 atmosphere. Finally the solid was filtered and washed with copious amount of toluene and water. The white solid was dried at 120 °C for 48 h. The resulting amine surface functionalized sample was denoted as 75-MBG-NH2 and 85-MBG-NH2, respectively.

2.3. Lysine functionalization of aminated 75/85-MBG-NH_2 samples

To provide the 75/85-MBG surfaces with zwitterionic nature, the materials were post-synthesis functionalized with Lysine (Lys) through glutaraldehyde (GA) linkage to amine groups. 1 g of 75/85-MBG-NH_2 was suspended in a solution of 2.5 mL of GA 50% w/v and 47.5 mL of deoxygenated water in a 100 mL round-bottom flask under an inert atmosphere to prevent oxidation. The mixture was stirred 1 hour at room temperature and an orange solid coloration was observed. The solid product was filtered and washed several times with water and subsequently added to a Lys-NHBoc aqueous solution (0.13 mg, (0.53 mmol), in 62.5 mL of deoxygenated water). The suspension was stirred overnight at room temperature. The orange solid was collected by filtration and washed exhaustively with water and then dried under vacuum. Finally, to check out Lys terminal amine, the orange solid was treated with trifluoroacetic acid (0.5 mL TFA in 20 mL dry dichloromethane) for 3 hours. The CH_2Cl_2 was removed by evaporation and the orange solid was suspended in water to wash by