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Fabrication and cytotoxicity assessment of novel polysiloxane/bioactive glass films for biomedical applications

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ABSTRACT

This work evaluates for the first time the cyto-compatibility of silicone (polysiloxane)/bioactive glass composite films produced by dip coating on stainless steel substrates using osteoblast-like (MG-63) cells. With the aim of creating corrosion resistant coatings for biomedical applications, bioactive glass (BG) of 45S5 composition was used as a filler in conjunction with commercial silicones (MK and H62C). Bioactive glass has the property of forming a direct bond to living bone, and polysiloxane is an attractive candidate for protective coatings due to its resistance to oxidation and corrosion. Suspensions based on polysiloxanes (MK/H62C) and micro-sized BG fillers were used for dip coating stainless steel substrates at room temperature, followed by curing in oxidative atmosphere at 260 °C and 500 °C. Fourier transform infrared spectroscopy (FTIR) analysis revealed the presence of Si–O–Si, Si–OR, Si–CH_3 and Si–OH groups on the substrate. Field emission scanning electron microscopy showed that the coatings were homogeneous with no obvious cracks or pinholes at relatively high concentrations of both polysiloxane and BG. The cell biology experiments confirmed that the expressed cell-morphology, analyzed on chosen surfaces, was pheno-typical for MG-63 cells after 48 h of incubation. On the film containing the lower amount of polysiloxane/BG the most dense cell layer was formed. Our results indicated that polysiloxane/BG composite films exhibited good cyto-compatibility at 260 °C and 500 °C and showed no toxicity toward MG-63 cells suggesting the potential of this composite for applications in medical implants.

1. Introduction

Research and development activities on composites of bioactive glass (BG) and polymeric materials, e.g saturated aliphatic polyesters (PLA, PGA, PCL) and natural biodegradable polymers (polysaccharides and proteins), have been underway for the last 2 decades aiming at developing systems that combine the bioactive character of BGs (in different chemical compositions) and the favorable mechanical properties (flexibility) of the polymers [1]. Among several polymeric materials, silicon-based preceramic polymers are interesting for engineering and chemical applications as they possess specific electrical, thermal, chemical and mechanical properties [2]. This class of materials is the ideal precursor for ternary and quaternary ceramics synthesized by controlled pyrolysis of the polymeric precursors referred to as polymer-derived ceramics (PDCs) [3]. Moreover, these polymeric precursors are intrinsically complex systems possessing excellent oxidation and creep resistance up to exceptionally high temperatures. The most known classes of PDCs are in the binary systems SiC, Si_3N_4, AlN, and BN, and ternary systems SiOC, SiCN, and BCN as well as in the quaternary systems SiBCO, SiBCN, SiCNO, SiAlCO, and SiAlCN [4–7].

Si-based preceramic polymers are organometallics that lose their organic components upon pyrolysis in a non-oxidative (inert) atmosphere leading to inorganic materials possessing improved and novel properties compared to conventional ceramics. Considerable work on polymer-derived ceramics has been mainly devoted to both producing porous structures with different compositions and controlling structures for functional and mechanical properties [5–7]. The advantages that offer the processing of ceramics and ceramic composites from polymeric precursors over traditional methods is the lower pyrolysis temperature and the ability to use polymeric processing techniques, such as dip coating and injection molding. The most promising area for the application of these materials has been in low-dimensional products such as coatings and as reactive binders to produce technical ceramics [4,8]. As developments in the field of biomaterials are promising for future applications of PDCs, the objective of this study is to

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explore the behavior of novel polysiloxane/bioactive glass composite materials with regard to their microstructural homogeneity and cyto-compatibility. Polysiloxane exhibits excellent abrasion and corrosion resistance, good chemical resistance, and is considered a good candidate for various applications, including protective coatings.

In 2012, Bernardo et al. [9] reported the manufacturing of calcium silicate ceramics from silicones with appropriate "active" and "passive" fillers. The formation of wollastonite–hydroxyapatite composites in air at 900 °C was optimized by the inclusion of hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) particles as "passive" fillers, together with $CaCO_3$ active filler. In vitro cell tests with human osteoblasts revealed the non-toxicity of the composite demonstrating the potential of filled silicones to fabricate biocompatible and bioactive materials for bone tissue engineering [9]. In the same context and as already mentioned in a previous investigation on wollastonite [10], the thermal treatment of silicone resins embedding micro and nano-sized particles of CaO and MgO at 900 °C led to the formation of akermanite ($Ca_2MgSi_2O_7$) ceramics. The addition of hydroxyapatite as additional filler in the synthesis of akermanite was shown to not only reduce the presence of cracks but also to modify the biological response of the produced components. Recent works have shown that bioactive wollastonite/apatite and wollastonite–diopside glass-ceramics can be obtained at 900–1200 °C from a mixture of silicone resin preceramic polymer and fillers such as calcium carbonate, zinc oxide, magnesium hydroxide and bioactive glass [11,12]. There are also previous studies reporting the slow degradation of Ca–Mg silicate in simulated body fluid (SBF) and its appropriateness for cell proliferation [13–15].

It appears that no consideration has so far been given to the cytotoxicity and biocompatibility of polysiloxane/bioactive glass composites obtained by dip coating and cured at low temperatures (up to 500 °C). The few available studies include works on the biocompatibility and phase evolution of polysiloxane at higher temperatures ($\geq$ 900 °C) after mixing with active or passive filler, e.g. apatite, calcium carbonate or magnesium carbonate [9,10,16,17]. Given the limited research results available, there is no conclusive information on the beneficial application of polymer-derived ceramics or silicon-based preceramic polymers in medicine. The intent of the present work is to gain insights on the cytotoxicity of polysiloxane/bioactive glass composites after crosslinking and curing at relatively low temperature (up to 500 °C). Bioactive glass (BG) of composition 45S5 (45% SiO_2, 24.5% CaO, 24.5% Na_2O, 6% P_2O_5 in wt%) was used, given that this BG exhibits high bioactivity and has been approved for clinical use [18].

On the other hand, ceramic coatings based on polysilazanes (Si–C–N system) in combination with borosilicate–barium silicate glass filler particle mixture were reported by Schütz et al. [19]. The passive glass fillers soften during the heat treatment of coated samples on steel substrates in air at 700 °C and form compact layers with closed pores. The properties of coatings that combine the advantages of ceramic layers and of glass coatings might be useful for steel protection from corrosion and abrasive wear at high temperatures.

Development of new corrosion resistant coatings is expected to open up a new era in the engineering of materials for medicine and especially for orthopedic and dental applications. The promise is that new composite films (polysiloxane/BG) deposited on biodegradable metals such as magnesium can provide not only bioactivity, e.g better bonding to bone, but also corrosion resistance of the implant for orthopedic applications.

2. Experimental

The precursors for SiOC are two different polysiloxanes, MK and H62C (SILRES® MK & SILRES® H62C Wacker–Chemie GmbH, München, Germany). SILRES® MK is a solid methyl polysilsesquioxane resin, characterized by a 84 wt% SiO_2 yield after pyrolysis in air, as specified by the manufacturer (typical curing conditions: 200 °C for 30–60 min). SILRES® H62C instead is a liquid polysiloxane, thermally cross-linkable without the formation of gaseous by-products, as specified by the manufacturer, as it contains very low content of volatile organic compounds (curing conditions: 170–200 °C for 30–60 min). The preceramic polymers were first dissolved in isopropyl alcohol by magnetic stirring for 30 min, then BG powder (size $< 10\ \mu m$) was added, for an overall solid content of about 11–20.5%. The BG micro-powder was thus dispersed into the solvent/silicones solution. Coatings were obtained by dipping the substrates (stainless steel foil) in the suspension (Silicone/ BG) for 3 cycles, followed by solvent removal at room temperature after each coating step, and subsequently by cross-linking at 260 °C to fix the coating on the substrate. Finally, the coated substrates were thermally cured in air at 500 °C for 60 min. Stainless steel (SS) 316 was chosen as the metallic substrate. Prior to processing, all SS316 substrates were cleaned using sonication, and dried in air.

Four different formulations (F) have been used in the dip coating experiments, as follows:

F1) 30 ml isopropanol + 10% MK + 1% BG
F2) 30 ml isopropanol + 5% MK + 4.2% H62C + 1% BG
F3) 30 ml isopropanol + 15% MK + 2% BG
F4) 30 ml isopropanol + 15% MK + 5% BG

The coated substrates were firstly cross-linked at 260 °C in air at a rate of 10 °C/min for a holding time of 30 min, followed by curing at 500 °C at a rate of 2 °C/min and for a holding time of 1 h. FTIR spectroscopy was performed in order to examine the functional groups present in the as-deposited films. Analysis was performed in reflectance mode with a JASCO instrument (FTIR-6300) in the wavenumber range 4000–400 cm^{-1}. The overall microstructural characterization of coated substrates after curing at 260 °C and 500 °C was performed using field emission scanning electron microscopy (FESEM, Quanta-FEG-250, Holland). A goniometer KSV CAM 200 system was used to perform the contact angle (θ) measurements for the uncoated substrate and for samples after dip coating of polysiloxane/BG composites. The contact angle was processed by an image analysis system, which calculated both the left and right contact angles from the shape of the drop.

To evaluate the cytotoxicity of MK (H62C)/BG coating, human osteoblast-like cells (MG-63) were employed. Samples of circular shape and a diameter of 10 mm were placed in 48 well plates (Greiner, Germany) for cell culture experiments. Four replicates of each sample were used. Before cell seeding the samples were sterilized at 121 °C in an autoclave (Systec, Germany). Cells were seeded onto the samples with density of 10^5 cells ml^{-1} for 48 h. Live staining of the cells on the samples was performed using Calcein (Life Technologies, Darmstadt, Germany). The cell distribution on the sample surface was analyzed using a fluorescence microscope (FM) (Axio Scope A.1, Carl Zeiss Microimaging GmbH, Germany).

3. Results and discussion

3.1. Wettability, chemical composition and microstructure

Fig. 1 illustrates the mean values of water contact angles (θ) after 4 measurements on stainless steel substrates coated with