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Indirect rapid prototyping of sol-gel hybrid glass scaffolds for bone regeneration – Effects of organic crosslinker valence, content and molecular weight on mechanical properties

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ABSTRACT

We present a series of organic/inorganic hybrid sol-gel derived glasses, made from a tetraethoxysilane-derived silica sol (100% SiO_2) and oligovalent organic crosslinkers functionalized with 3-isocyanatopropyl triethoxysilane. The material was susceptible to heat sterilization. The hybrids were processed into pore-interconnected scaffolds by an indirect rapid prototyping method, described here for the first time for sol-gel glass materials. A large panel of polyethylene oxide-derived 2- to 4-armed crosslinkers of molecular weights ranging between 170 and 8000 Da were incorporated and their effect on scaffold mechanical properties was investigated. By multiple linear regression, 'organic content' and the 'content of ethylene oxide units in the hybrid' were identified as the main factors that determined compressive strength and modulus, respectively. In general, 3- and 4-armed crosslinkers performed better than linear molecules. Compression tests and cell culture experiments with osteoblast-like SaOS-2 cells showed that macroporous scaffolds can be produced with compressive strengths of up to 33 ± 2 MPa and with a pore structure that allows cells to grow deep into the scaffolds and form mineral deposits. Compressive moduli between 27 ± 7 MPa and 568 ± 98 MPa were obtained depending on the hybrid composition and problems associated with the inherent brittleness of sol-gel glass materials could be overcome. SaOS-2 cells showed cytocompatibility on hybrid glass scaffolds and mineral accumulation started as early as day 7. On day 14, we also found mineral accumulation on control hybrid glass scaffolds without cells, indicating a positive effect of the hybrid glass on mineral accumulation.

Statement of Significance

We produced a hybrid sol-gel glass material with significantly improved mechanical properties towards an application in bone regeneration and processed the material into macroporous scaffolds of controlled architecture by indirect rapid prototyping. We were able to produce macroporous materials of relevant porosity and pore size with compressive moduli, covering the range reported for cancellous bone while an even higher compressive strength was maintained. By multiple linear regression, we identified crosslinker parameters, namely organic content and the content of ethylene oxide units in the hybrids that predominantly determined the mechanics of the hybrid materials. The scaffolds proved to be cytocompatible and induced mineralization in SaOS-2 cells. This provides new insight on the critical parameters for the design of the organic components of covalent hybrid sol-gel glasses.

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1. Introduction

The field of bioactive glasses has expanded strongly since the discovery of 45S5 Bioglass® by Larry Hench [1,2]. The 45S5 Bioglass® composition was a great success and is used mainly in particulate form for dental applications [3]. Several melt-derived glasses have been developed, such as S53P4 [4] and 13–93 compositions [5], to increase crystallization temperature and optimize mechanical performance. Later, sol-gel glasses offered the possibility to synthesize bioactive glasses, which do not require sintering and thus remain amorphous. They also have a large surface area due to their mesoporosity [6,7], which results in good cell adhesion and osteogenic differentiation [8]. Nevertheless, all purely inorganic glasses are characterized as brittle [9], which is a great disadvantage for the purpose of bone regeneration. In order to produce mechanically competent structures, it is necessary to overcome the brittleness of inorganic glasses, which can be accomplished by the production of hybrid glasses [10] and composite materials containing bioactive glass particles [11]. Composite materials however, show specific disadvantages, due to their heterogenic nature, which have been discussed in literature [2], such as different degradation rates of the two components, or the polymer matrix masking the effect of the glass particles. The sol-gel-process yields a homogeneous material and the mechanical properties of the resulting bioactive glass can be modified by the introduction of organic crosslinkers, which form a covalently linked organic-inorganic interpenetrating network [12–14]. Popular crosslinkers are biopolymers such as poly(glutamic acid) [15], gelatin [16,17] and smaller poly- or oligomers such as polyethylene glycol (PEG) [18], polycaprolactone (PCL) [19] and polyvinyl alcohol (PVA) [20]. In this study, we used 2- to 4-armed organic crosslinkers and intended to determine the impact of crosslinkers' size and content in the hybrid sol on the mechanical properties. To this end, we chose small organic core molecules from 170 Da to 8000 Da with 2, 3 and 4 hydroxy-terminated arms, which were functionalized with 3-isocyanatopropyltriethoxysilane, to crosslink a pure, tetraethoxysilane (TEOS)-derived silica sol. From the hybrid material macroporous scaffolds were produced and the influence of crosslinkers' parameters on the mechanical properties of the scaffold was assessed.

For guided tissue regeneration of large bone defects, a scaffold has to bridge a large gap, making three-dimensional, solid scaffolds necessary. These scaffolds also need to provide an open macroporosity for cell infiltration and vascularization. From melt-derived glass particles, such scaffolds can be fabricated via the polymer foam replication technique with the disadvantage that the high porosity and thin glass struts cause a relatively low mechanical strength [21]. The development of the sol-gel process provided new ways to produce three-dimensional scaffolds more easily, by allowing for templating techniques such as direct foaming [22,23], thermal decomposition of organic fillers [24], electrospinning [25], fiber pulling [26], salt leaching [27], robotic deposition [28] and a combination of rapid prototyping, dip coating and thermal decomposition of the template [29]. However, none of these templating techniques led to the production of organic-inorganic hybrid scaffolds with a defined pore structure. This is mainly because the material has to be sintered when processed by robotic deposition techniques, which would burn out any organic crosslinker. For non-robotic templating techniques, control over pore size and pore geometry may be limited. A templating technique that would solve these limitations is indirect rapid prototyping, which has been used for different materials [30–36], but not yet for silicate glasses. Significant advantages of this method are (1) excellent control over the scaffold's shape, pore geometry and –interconnectivity and (2) the possibility to produce scaffolds from a large pool of materials [30]. In this study polycaprolactone (PCL) templates were prepared and used as lost molds to form macroporous scaffolds from a variety of hybrid silica sol compositions. Because the template can be easily leached, once the sol has gelled, the scaffolds do not have to be strongly heated and could be produced in any geometry with a pore structure defined by the PCL template.

Nuclear magnetic resonance was used to characterize the silica sol and the organic crosslinker, the morphology of scaffolds was analyzed by scanning electron microscopy, the mechanical properties of the scaffolds were determined via compression tests and the biocompatibility was tested *in vitro* with SaOS-2 cells.

We proposed that with indirect rapid prototyping a large range of organic/inorganic hybrid sols can be processed to scaffolds with large, interconnected pores with good mechanical properties for bone regeneration. To this end, we studied the effect of the crosslinker on the mechanical properties and assessed the biological activity by *in vitro* cell culture with SaOS-2 cells.

2. Experimental

2.1. Materials

Ethanol 96% (v/v) and calcium chloride dihydrate were purchased from AppliChem (Darmstadt, Germany). Deuterated dimethyl sulfoxide (d_6-DMSO) was purchased from Armar Chemicals (Leipzig, Germany). Poly(ethylene glycol)s (PEG 200 (average $M_n \sim 200$), PEG 600 (average $M_n \sim 600$), PEG 1450 (average $M_n \sim 1450$), PEG 4000 (average $M_n \sim 4000$), PEG 8000 (average $M_n \sim 8000$)) and 3-isocyana topropyltriethoxysilane (ICPTES) were purchased from Fisher Scientific (Schwerte, Germany). Polycaprolactone (PCL, $M_n \sim 45{,}000$), trimethylolpropane ethoxylates (TMPEO 170 (average $M_n \sim 170$), TMPEO 450 (average $M_n \sim 450$), TMPEO 730 (average $M_n \sim 730$), TMPEO 1014 (average $M_n \sim 1014$)), pentaerythritol ethoxylates (PETEO 270 (average $M_n \sim 270$) and PETEO 797 (average $M_n \sim 797$)) and 1,4-diazabicyclo[2.2.2]octan (DABCO), Dulbeco's modified Eagle's medium (DMEM), McCoy's medium, dexamethasone, β-glycerolphosphate, ascorbic acid-2-phosphate, ALP-substrate and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich (Seelze, Germany). Tetrahydrofuran (THF) was purchased from VWR International (Dresden, Germany). PicoGreen® and Alexa Fluor® 488 Phalloidin were purchased from life technologies (Darmstadt, Germany). PCL-scaffolds used as controls in cell culture were made by 3D-Biotek (catalog No. PCL303096-24) and ordered from Sigma-Aldrich.

2.2. Methods

2.2.1. Template fabrication

Templates for the scaffold fabrication were produced from PCL via rapid prototyping (Fused Deposition Modeling – FDM) using a Bioscaffolder® (SYSENG). The polymer was melted at 75 °C and extruded through a needle with an inner diameter of 0.178 mm in a meandering pattern to produce a cylinder with a layered grid structure. The line spacing in x-y levels was set to 0.43 mm and the level spacing in z was set to 0.14 mm. Two layers of PCL-strands were extruded on top of each other in the same orientation before the orientation was rotated 90°. The cylinders had a closed circumferential surface with pores only accessible from the top and the bottom (Fig. 1A). The cylinders measured 7.9 × 8 mm (d × h) (7 mm inner diameter).

2.2.2. Silicate sol synthesis

The silicate sol was produced by mixing 217.5 g tetraethoxysilane (TEOS) and 60.0 g ethanol before adding 32.2 g of 0.05 M H_2SO_4 to yield a molar ratio of H_2O to TEOS of 1.78:1. The solution was stirred at 55 °C for 18 h before 188 g of ethanol were evaporated