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Fabrication, multi-scale characterization and in-vitro evaluation of porous hybrid bioactive glass polymer-coated scaffolds for bone tissue engineering

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ABSTRACT

Bioactive glass-based scaffolds are commonly used in bone tissue engineering due to their biocompatibility, mechanical strength and adequate porous structure. However, their hydrophobicity and brittleness limits their practical application. In this study, to improve nanomechanical properties of such scaffolds, pure bioactive hybrid glass and two bioactive hybrid glass-polymer coated composites were fabricated. A complementary micro and nanoscale characterization techniques (SEM, AFM, μCT, FTIR, compressive test, goniometer) were implemented for detailed description of architecture and physicochemical properties of hybrid bioactive glass-based scaffolds with emphasis on nano-mechanics. The final step was in-vitro evaluation of three dimensional macroporous structures. Our findings show that after polymer addition, architecture, topography and surface properties of the scaffolds were changed and promoted favoured behaviour of the cells.

1. Introduction

Nowadays there are more than million patients annually in the World, suffering from bone loss or failure resulting from an injury, cancer removal, bone infections or other type of disease. The problem is also progressive loss of bone density with age [1–4]. Although the bone can repair itself and regenerate in case of small damages, it fails in large and massive defects or pathological fractures. Most common treatment methods in such cases are based on implantation of bone grafts or permanent implants. Among others disadvantages of previously applied solutions in bone surgery the main are: donor shortage or risk of infection in case of transplantation, wear of synthetic materials, cytotoxicity and corrosion of metallic objects [5–8].

In the scope of increasing number of patients needing an implantation, the development of novel, biocompatible and cheap materials is crucial. For over 60 years, a revolution in bone surgery has been underway based on the introduction of new biocompatible materials and the design of an appropriate implant structures. The first generation of non-toxic biomaterial scaffolds were inert structures, which after the implantation did not interact with the surrounding tissues. The second generation included bioactive materials, on surface of which biochemical reactions took place to connect them with neighbouring tissues. Currently, the third-generation of biomaterials being in use, additionally mimics the naturally occurring structures, like extracellular matrix (ECM). Their role is to replace the damaged body fragment or supplement the tissue loss, as well as to stimulate living cells to grow and regenerate [9–15].

The biomaterials used for the curing of mechanically damaged or unwell bone tissue should be: inexpensive, sterilizable, having physical and chemical properties compatible with the host tissue, biocompatible (not provoking abnormal inflammatory or immunologic responses, non-allergic, non-cancerogenous). Additionally, scaffold material should have an adequate mechanical properties (strength, stiffness, fatigue properties), porosity, osteoconductivity, appropriate density, manufacturability, long-term storage [16–18].

Main objective of this study was to find, develop and obtain a good candidate for a bone scaffold material with taking into account the results of previous research. A good example of scaffold material meeting the criteria listed above are bioactive glass-based porous foams, which due to their biocompatibility and adequate porous structure are widely studied for bone tissue regenerative properties [19–22]. The disadvantage of these foams is their brittleness and hydrophobicity [23,24]. Such flaws contribute to the loose placement of the implant inside the patient's body, which consequently prevents its

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connection to the bone. Unfortunately, in the case of bioactive materials, the bone loss can regenerate only on the condition of close adherence and immobilization of the implant. To eliminate these problems, it was proposed to use materials with low modulus of elasticity, which are mostly polymeric materials [25]. To capitalize on their advantages and minimize their shortcomings, bioactive glass porous foams can be combined with polymers. For that purpose, the aim of this study was to evaluate the influence of polymer coating on nanomechanical properties, architecture and topography of bioactive glass foams. To the best of our knowledge, this is the first study in which mechanical properties of porous hybrid bioactive glass polymer-coated bone tissue engineering scaffolds were evaluated in the nanoscale.

2. Materials and methods

2.1. Materials for synthesis

Tetraethylorthosilicate (TEOS, 98%) and triethoxyvinylsilane (TEVS, 97%) were purchased from Sigma-Aldrich, and used without further purification. Calcium dichloride dehydrate ($CaCl_2{\cdot}2H_2O$) was purchased form Sigma-Aldrich. An aqueous solution of 0.01 M hydrochloric acid was prepared from 37% HCl (Chempur). Ethanol (absolute) was purchased from J. T. Baker. Ammonium carbonate ($(NH_4)_2CO_3$) was purchased from POCh. White granulated sugar (Diamant) was purchased from Pfeifer & Langen Marketing, and was used as received.

2.2. Fabrication of the scaffolds

2.2.1. Synthesis of the organic-inorganic hybrid bioactive glass

Hybrid bioactive glass was prepared using the sol-gel process. In a typical synthesis, silanes TEOS (20 mL) and TEVS (20 mL) were mixed in 1:1 volume ratio and dissolved in a mixture of anhydrous ethanol (20 mL) and an aqueous solution of 0.01 M HCl (10 mL). Hydrochloric acid was used as a catalyst for the hydrolysis and further condensation reactions. To obtained solution, 25 mol% of $CaCl_2{\cdot}2H_2O$ was added. The mixture was polymerized by heat treatment at 70 °C in the presence of commonly used initiator in radical polymerization – *tert*-butyl hydroperoxide. Next, the resulting solution was stirred for 5 h and then cooled down to room temperature and the sol was left overnight stirring.

2.2.2. Preparation of the macroporous scaffolds

Macroporous scaffolds (herein designated as HGV_PURE) were prepared using slightly modified procedures described in the literature [26–30]. As a template for macroporous scaffold white granulated sugar mixed with ammonium carbonate (3–5 wt%) was used. In this case, porogen was closely packed in cylindrical molds (high, 2 cm; radius 1 cm). Next, the obtained sol was evaporated under vacuum to obtain dense solution (viscosity ca. 1500 cP). Then, the sugar template was moistened with evaporated hybrid glass and heated at 60 °C for 3 days until the gelation process occurred. The porogen was then washed out using warm distilled water (40–50 °C) until the filtrate had no traces of sugar. The presence of sugar was examined using the Molisch (α-naphthol) test [31]. In a standard procedure, 0.5 mL of the filtrate was mixed with 5 mL of cold 75% H_2SO_4. Three drops of a 3% α-naphthol solution in ethanol were added to the acid mixture. A yellow color was produced by the addition of the naphthol. After this, the mixture was warmed up on a water bath at 80 °C. Depending on the amount of carbohydrates, a red to blue violet color appeared throughout the whole mixture. In the absence of carbohydrates, the examined solution remained yellow. After porogen removal, resulting gel was aged at 120 °C for 5 days.

2.2.3. Preparation of the hybrid glasses covered by polyesters

Obtained macroporous hybrid bioactive glasses were covered with biodegradable polymers using polylactide (namely HGV_PLA) and polycaprolactone (HGV_PCL). For this purpose, the polymers were dissolved in dichloromethane (1 g of polylactide or polycaprolactone was dissolved in 50 mL of dichlomethane). The resulting solution was poured into containers with previously prepared scaffolds. The preparation of the polymer-covered scaffolds consisted of pumping the air out of the macropores, acting vacuum for 5 h and simultaneously heating the vessel from the room temperature to 50 °C. After immersing of outgassed scaffolds into the polymer solution, the solvent was slowly evaporated (after 3 h immersion) to form the materials coated with a polymeric film.

2.3. Scanning electron microscopy

The morphology of scaffolds was registered with scanning electron microscopy (SEM) (Phenom Pro X, FEI) equipped with special holder designed for non-conductive samples. Back Scattered Electron mode was used with applied voltage of 5 kV. Due to the complex architecture and brittleness of the material, samples were not coated prior to imaging.

2.4. Atomic force microscopy

2.4.1. Topography

Hybrid glass-based scaffolds visualization was made with Multimode 8 (Bruker/Veeco), equipped with Nanoscope 5 controller. To register topographical images, silicon ACST type (APP NANO) scanning probe was used. The nominal spring constant of the probe was 7.8 N/m and the radius of the probe was below 10 nm. Drive frequency (ca. 150 kHz) of the scanning probe was adjusted using Auto Tune method. Scaffolds imaging (twenty five randomly selected areas) was conducted in Tapping Mode in the air, under ambient conditions. Analysis of registered topographical images was conducted with use of NanoScope Analysis (ver. 1.40).

2.4.2. Elastic modulus and adhesion force evaluation

Elastic modulus of the surface of synthesized scaffolds was measured along with adhesion force between scanning probe and samples' surface using Multimode 8 (Bruker/Veeco), equipped with Nanoscope 5 controller. ACST type (APP NANO) scanning probe with the tip radius below 10 nm and spring constant of 7.8 N/m was installed in the microscope. AFM operating in the Quantitative NanoMechanics Mode (QNM) allowed registration of stiffness and adhesion forces maps (twenty five randomly selected areas). Measurements were performed in the air, under ambient conditions (temp. of 21 °C and relative humidity of 18%). Each map was composed of 256 × 256 pixels, from which so called force-distance curves were collected. Analysis of registered nanomechanical data was done with NanoScope Analysis (ver. 1.40), professional, dedicated software provided by the manufacturer of the microscope.

2.5. Computer micro tomography (μCT)

Scanning was performed with use of the Skyscan 1172 micro CT X-ray microtomograph (Bruker microCT) equipped with Hamamatsu 100/250 X-ray tube and SHT 11 Mp X-ray camera. The samples prepared for μCT measurement were stacked one on the scanner sample holder. The μCT scans for all samples were obtained in the cone X-ray beam acquisition. The acquired dataset consisted of 3000 rotation image 2D-vertical-projections in 16-bit TIFF format (4000 × 2664 pixels) for all the samples. The scanning process took about 180 min and was carried out at constant temperature of 20 °C. For each sample, the 3D cross sectional image stack was reconstructed from the rotation image 2D vertical projections, using the NRecon cone X-ray beam reconstruction software (Bruker microCT), based on the Feldkamp algorithm. The NRecon software allowed adjusting four of the reconstruction parameters: smoothing, post-alignment, ring artifact