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Polyurethane foam/nano hydroxyapatite composite as a suitable scaffold for bone tissue regeneration

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ABSTRACT

In bone tissue regeneration, the use of biomineralized scaffolds to create the 3D porous structure needed for well-fitting with defect size and appropriate cell interactions, is a promising alternative to autologous and heterologous bone grafts. Biomineralized polyurethane (PU) foams are here investigated as scaffold for bone tissue regeneration. Biomineralization of the foams was carried out by activation of PU surface by a two steps procedure performed for different times (1 to 4 weeks). Scaffolds were investigated for morphological, chemico-physical and mechanical properties, as well as for in vitro interaction with rat Bone Marrow Mesenchymal Stem Cells (BMSCs). Untreated and biomineralized PU samples showed a homogenous morphology and regular pore size (average Ø = 407 μm). Phase and structure of formed calcium phosphates (CaPs) layer onto the PU foam were analyzed by Fourier Transform Infrared spectroscopy and X-ray diffraction, proving the formation of bone-like nano hydroxyapatite. Biomineralization caused a significant increase of mechanical properties of treated foams compared to untreated ones. Biomineralization also affected the PU scaffold cytocompatibility providing a more appropriate surface for cell attachment and proliferation. Considering the obtained results, the proposed scaffold can be considered suitable for bone tissue regeneration.

1. Introduction

Due to the large number of patients suffering of bone defects caused by trauma, tumor or diseases, in recent years, numerous investigations are in progress, making efforts on the development of new materials [1–4] and processing techniques [5,6] for bone tissue regeneration. Nevertheless, in spite of the intense researches, there is a big gap between the ongoing in vitro studies and the clinical innovative approaches. Nowadays, in clinical therapies for bone regeneration, autologous bone, allografts, demineralized heterologous bone or bone substitutes are used. Although autografts represent an excellent option, thanks to their osteoconductivity, osteoinductivity and non-immunogenicity [7], their use is limited by donor shortage and donor site morbidity [8]. In addition, allograft presents the risk of immunological problems and disease transmission [9]. Therefore, a bone defect reconstruction can greatly benefit from alternative sources, especially from engineered scaffolds with the capability of integration into the surrounding bone tissue. In order to allow the regeneration of a natural bone tissue, the scaffold should possess a suitable surface chemistry to support cell adhesion, proliferation, migration and growth. In addition, it should act as a biocompatible template for osteoprogenitor cell growth, promote the differentiation of mesenchymal stem cells (MSCs) into osteoblast phenotype and support production, organization and maintenance of the extracellular matrix. Finally, scaffolds are required to have highly interconnected pores with adequate size to promote cell migration and nutrient distribution [10]. The use of natural or synthetic polymers for bone tissue regeneration is extremely appealing due to the possibility of processing them into three-dimensional (3D) structures [11]. Collagen [12], chitosan [13], gelatin [14], hyaluronic acid [15] and alginate [13] represent the most promising natural polymers for bone tissue regeneration. However, despite their excellent biocompatibility, the use of such polymers by themselves is limited by significant drawbacks such as the weak mechanical properties and poor processability. To improve the mechanical properties of the structure,

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composite scaffolds based on natural polymers as matrix and reinforced with inorganic particles are under investigation [16–18,61]. Synthetic biodegradable polymers are proposed for bone tissue regeneration as well, including polydioxanone [19], polyorthoesters [20–23], polyanhydrides [19], poly (α-hydroxyesters) [20–23], poly (lactide-*co*-glycolide) [21–23] and polyurethanes [4,24]. However, balancing between in vivo degradation and tissue regeneration is not easily achievable, because it depends on different variables in clinical conditions, such as shape and size of bone defect, release of acidic degradation products that may lead to non-physiological inflammatory response, and functional loading, which affects bone regeneration and remodeling.

An interesting alternative to biodegradation is biointegration. It can be obtained by using polymers with a slow degradation rate, specifically designed to fulfil all the key requirements. Scaffolds could be effectively used to restore large defects in such a way to prevent tissue collapse and sustain the newly forming tissue for longer time than conventional biodegradable polymers. In this perspective, polyurethanes (PU) show a range of mechanical and morphological properties significantly larger than other medical-grade biodegradable polymers [4,25,26]; in particular, promising results have been obtained with polyurethane foams both in vitro [27–31] and in vivo animal models (rat, sheep) [28,32] for bone tissue regeneration. In the last years, crosslinked PU foams with slow degradation rate and controlled range of pore size, open porosity and mechanical properties were developed [4,33]. PU foams with different range of hydrophilicity [34], surface modified with proteins [35] and composites [34] have been investigated.

According to some researches [36,37], one of the important requirements for a synthetic material to show a bone-bonding behaviour is the formation of a calcium phosphate interface similar to bone apatite. On the other hand, the presence of an apatite-like layer on the scaffold surface is the sign of a positive biological response from the host tissues. Hence, it is expected that a material holding that kind of coating would present a bioactive behaviour after implantation [38]. Bioactive glass is such a material that has the ability to form an apatite-like layer on the polymer surface and therefore bonding to living tissues [39]. In the formation mechanism of this layer, silanol groups play an important role [40,41]. Several studies [42–44] report investigation for mimicking the in vivo natural processes leading to CaPs deposition so to allow an in vitro mineral phase deposition onto polymeric 3D porous scaffolds. However, the main problem is providing the adequate chemical conditions on the substrate [42–44], so to induce the precipitation of CaPs phase [38,62,63]. CaPs coatings have been produced on a different kinds of materials such as metals [42,43], non-biodegradable polymers [43,44], bioinert ceramics [43], and even natural polymers like bamboo [45].

Polyurethanes can undergo calcification in vivo [46] and, if this represents an important problem for cardiovascular applications, urinary prosthesis and intrauterine contraceptive devices, it becomes beneficial for bone substitution. In fact, the deposition of CaPs onto the polyurethane surface can promote osteoconductivity and bone bonding [47].

In the present study, a treatment based on calcium and phosphate ions as precursors for the nucleation and growth of calcium phosphate on the pore wall of PU porous scaffold was performed (Fig. 1) and investigated. After that, the adhesion and proliferation of Bone Marrow Mesenchymal Stem Cells (BMSCs) isolated from rat femora and tibia, were investigated on the biomineralized PU foam.

2. Materials and methods

2.1. Polyurethane foam synthesis

The poly-ether-urethane (PU) foam was synthesized according to a previously optimized production process [4,34,48]. The synthesis process consisted in a one-step bulk polymerization, performed by gas foaming reaction. The reaction mixture was prepared in a polypropylene beaker by adding an ad hoc prepared poly-ether-polyol mixture [49,59], distilled water as expanding agent (2% w/wpolyol), Fe-acetylacetonate as reaction catalyst (FeAA, 0.001% w/wpolyol) and 4-4′-methylene diphenyl diisocyanate prepolymer (Desmodur PF, Bayer Germany, NCO = 5.476 mmol/g). The synthesis was performed using a non-stoichiometric ratio of OH/NCO = 100/133, with an isocyanate excess. The reaction mixture was mixed by a mechanical stirrer (ALCW750, MAVER) at 2000 rpm for 1 min. After mixing, 75 g of the reaction mixture were poured inside a custom-made poly (methyl methacrylate) mold (V = 500 cm^3, Fig. 1). The mold was firmly tight so to allow a confined expansion process, thus obtaining foams homogeneously expanded, with controlled and reproducible properties. The mold was kept at room temperature (R.T.) for 72 h to allow for the complete gas foaming reaction. At completed reaction, the foam was manually removed from the mold; the compact external skin (thickness = 1 cm) was gently removed, so to obtain a homogeneous porous structure. The foam was finally post-cured at R.T. for 3 days. For morphological, physical and mechanical characterization and in vitro biological investigation tests, cylindrical samples (Ø = 10 mm, h = 4 mm) were obtained by manually punching PU foam slices. Samples were immersed in pure ethanol for 48 h, to allow the complete removal of possible low molecular weight products that could affect the PU foam cytocompatibility, and subsequently let dry at R.T. for 24 h before further characterization.

2.2. Biomineralization process

Biomineralization treatment (Fig. 1) was performed on PU foam specimens as substrates for nucleating the apatite film. PU samples were immersed in $CaCl_2 \cdot 2H_2O$ (Sigma-Aldrich) 0.5 M for 3 days at 37 °C and after washing with distilled water, soaked in $Na_2HPO_4 \cdot 12H_2O$ (Sigma-Aldrich) 0.3 M for 3 days at 37 °C. The aim of this step was the initial nucleation of calcium phosphate layer due to a chemical reaction between Ca and P ions on the PU scaffold surface. After that, biomineralization was carried out by immersion of the PU samples in 1.5 SBF for different time points (t = 1, 2, 3 and 4 weeks). 1.5 SBF contains a concentrations of Ca^{2+} and PO_4^{3-} ions 1.5 times larger than SBF that presents an ion concentration nearly equal to human blood plasma. The 1.5 SBF solution was prepared using the Kokubo et al. [43] protocol, by dissolving NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 (Sigma-Aldrich) in distilled water (Table 1), buffered with tris (hydroxymethyl) aminomethane, $(CH_2OH)_3CNH_2$, and adjusting pH at 7.4 at 37 °C by stirring the solution and titrating it with HCl. Each week, the 1.5 SBF solution was changed to preserve 1.5 SBF constant ion concentrations during the nucleation procedure. Four group samples (n = 20 each) were prepared considering different immersion times in 1.5 SBF (1, 2, 3 and 4 weeks) and named PU-1W, PU-2W, PU-3W and PU-4W, respectively.

2.3. Scaffold morphological characterization

For morphological evaluation, PU foam specimens before and after HA nucleation treatment were mounted on aluminum stubs, gold sputter-coated (Sputter Coater S150B, Edward) and observed by Scanning Electron Microscopy (SEM, StereoScan 360 Cambridge) at 10 kV. Deeper investigation on the morphology of nucleated calcium phosphate layer on PU foams at the considered time-points was performed by SEM (KYKY-EM3200) at 26 kV.

2.4. Physical characterization

For physical characterization, density and water uptake tests were carried out. Density analysis was performed on untreated and treated PU foam specimens (Ø = 10 mm, h = 4 mm, n = 5). EN ISO 845