βTCP-Poly(3hydroxybutyrate) for Bone Tissue Engineering

Subjects: Others

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Tissue engineering is a technique that involves the in vitro seeding and attachment of cells onto a three-dimensional scaffold. In the case of bone tissue engineering, investigations have been focused mostly on synthetic bioceramic scaffolds based on calcium phosphates, such as hydroxyapatite or tricalcium phosphate. Due to their chemical similarity to an inorganic component of bone, hydroxyapatite, as well as βTCP- and αTCP-based materials, show excellent biocompatibility and osteoconductivity.

β tricalcium phosphate | silver-decorated scaffolds | P(3HB) coating | P(3HB) degradation | bacterial polymer

1. Overview

Implantations in orthopedics are associated with a high risk of bacterial infections in the surgery area. Therefore, biomaterials containing antibacterial agents, such as antibiotics, bactericidal ions or nanoparticles have been intensively investigated. In this work, silver decorated β tricalcium phosphate (βTCP)-based porous scaffolds were obtained and coated with a biopolymer—poly(3-hydroxybutyrate)-P(3HB). To the best of our knowledge, studies using silver-doped βTCP and P(3HB), as a component in ceramic-polymer scaffolds for bone tissue regeneration, have not yet been reported. Obtained materials were investigated by high-temperature X-ray diffraction, X-ray fluorescence, scanning electron microscopy with energy dispersive spectroscopy, hydrostatic weighing, compression tests and ultrahigh-pressure liquid chromatography with mass spectrometry (UHPLC-MS) measurements. The influence of sintering temperature (1150, 1200 °C) on the scaffolds' physicochemical properties (phase and chemical composition, microstructure, porosity, compressive strength) was evaluated. Materials covered with P(3HB) possessed higher compressive strength (3.8 ± 0.6 MPa) and surgical maneuverability, sufficient to withstand the implantation procedures. Furthermore, during the hydrolytic degradation of the composite material not only pure (R)-3-hydroxybutyric acid but also its oligomers were released which may nourish surrounding tissues. Thus, obtained scaffolds were found to be promising bone substitutes for use in non-load bearing applications

2. Calcium Phosphate

Tissue engineering is a technique that involves the in vitro seeding and attachment of cells onto a three-dimensional scaffold. In the case of bone tissue engineering, investigations have been focused mostly on synthetic

bioceramic scaffolds based on calcium phosphates, such as hydroxyapatite or tricalcium phosphate. Due to their chemical similarity to an inorganic component of bone, hydroxyapatite, as well as βTCP- and αTCP-based materials, show excellent biocompatibility and osteoconductivity. To further enhance their physicochemical and biological characteristics modifications with monovalent (Ag^+, Na^+), divalent (Mg^{2+}, Zn^{2+}) and trivalent (Fe^{3+}) metal ions were done [1][2][3]. Due to the high risk of bacterial infections during implantation procedures, biomaterials containing antibacterial agents, such as antibiotics, bactericidal ions (e.g., Ag^+, Au^+, Cu^{2+}) and nanoparticles have been intensively investigated. Recently, silver-modified biomaterials have been prepared by various methods that, *inter alia*, involve the incorporation of silver ions into their structure or attachment of silver nanoparticles on their surface. Silver-containing tricalcium phosphate microspheres composed of α/βTCP phases were synthesized using an ultrasonic spray-pyrolysis technique [4]. Su et al. [5] studied silver containing calcium phosphate coatings on pure iron foam obtained via co-deposition and post-treatment method. Siek et al. [6] used wet chemical method to obtain bactericidal αTCP-based bone cements with silver-modified hydroxyapatite (Ag-HA) and $CaCO_3$. It has been found that bone cement matrix did not impede the Ag^+ ions release from the Ag-HA agglomerates and antibacterial activity depended on the kind of bacterial strain. Hoover et al. [7] obtained silver-modified porous β-tricalcium phosphate (βTCP) scaffolds using liquid porogen based method. In their work silver added in the amount between 0.5 and 2 wt.% Ag_2O could be released over a long period of time without compromising the biocompatibility of the scaffolds. The bactericidal activity was achieved due to sustained release of Ag^+ ions through the continuous dissolution of Ag-modified βTCP. Materials for various biomedical applications, including chitosan [8], cellulose and its derivatives [9][10] as well as mesoporous carbons [11] decorated with silver nanoparticles were also examined. However, silver decorated calcium phosphate-based scaffolds seem to be an interesting and not yet fully explored area.

Calcium phosphate (CaP)-based bioceramic scaffolds are inherently brittle and often cannot match the mechanical properties of the bone. Thus, composites made of CaPs and bioresorbable polymers have also been investigated [12][13]. Recently, many researchers have been working on the fabrication of polymer-coated bone scaffolds including ceramic-biopolymer hybrid systems [14][15]. A new trend in the development of biomedical composites is a usage of polyhydroxyalkanoates (PHAs)—the bacterial derived polymers [16][17]. Under normal growth conditions, most bacteria produce only a small amount of PHA (1–15%). When special growth conditions and fermentation strategies are applied, the synthesized PHA can reach almost 90% [18]. Medical applications require a constant and reproducible quality of PHAs, which can be achieved via bacterial production in a rigorous culture [19]. Polyhydroxyalkanoates are a diverse group of materials with different applications and properties. It is known that more than 150 types of PHAs can be synthesized by microorganisms [20]. The material characteristics associated with PHAs are affected by many parameters including their chemical structure and type of monomer units with molecular mass. PHAs can be classified into short chain length (scl) PHAs with 4–5 carbons in pending monomers backbone or medium chain length (mcl) PHAs with (6–14 carbon atoms), when aliphatic monomers are present, and may further be grouped a homo-polymers (either scl-PHAs or mcl-PHAs) or copolymers (a mixture of different monomers of scl-PHA and/or mcl-PHA monomers). There were many attempts to use PHAs in medical applications due to their excellent biocompatibility and biodegradability [21][22][23][24]. PHAs have an advantage over other bioplastics such a poly(lactic acid) (PLA) or poly(D,L-lactide-co-glycolide) (PLGA) since their monomers (3-

hydroxylated acids) are quickly metabolized within the human body. Furthermore, they can be naturally detected in almost all parts of the body as a degradation product and were found not to cause carcinogenesis during long-term implantation [25][26]. Among the PHA family, the P(3HB) is the most common and well-characterized scl-PHA. The possibility of using P(3HB) as coating materials for composite type inorganic-organic scaffolds was demonstrated among others by Montazeri et al. [27]. It has been found that scaffold made of bioglass covered with polyhydroxybutyrate (PHB) possessed higher mechanical strength and bioactivity than nano-bioglass strut alone. In this study, to improve the mechanical properties and surgical maneuverability of brittle silver decorated βTCP scaffolds, they were covered with bioresorbable P(3HB) polymer. These kinds of highly-porous materials may be potentially applied for filling small bone defects in non-load or low-load bearing places. In the future the P(3HB) coating may serve as a drug delivery vehicle. Development of novel methodologies to fabricate bioceramic-PHAs composites may open new horizons for their applications in medicine. Therefore, silver decorated calcium phosphates, covered by a PHA layer seem to be interesting materials that have not yet been explored. This proof-of-concept study delivers some insights into the synthesis of such materials and their characterization, setting a benchmark for further developments of material for regenerative medicine based on biopolymers and bioceramics.

3. Conclusions

The macroporous β-tricalcium phosphate scaffolds modified with silver (Ag-βTCP) were prepared by a polyurethane foam replica method, followed by the coating of bacterially derived polymer-P(3HB). Materials with open porosity between 61.8 ± 3.0%–68.1 ± 4.6% and pore sizes in the range of 100–700 µm, were obtained. Concerning the consolidation of bioceramic struts, an appropriate sintering regime was achieved at 1150 °C, which is reflected in the highly dense microstructure with a small amount of microcracks. In higher temperatures, exceeding the critical grain size resulted in regions with local intergranular cracks. The compressive strength of scaffolds was in the range from 2.1 ± 0.6 to 3.8 ± 0.6 MPa, which is comparable to the compressive strength of spongy bone and can be sufficient for implantation in the low-load-bearing places. The designed scaffolds cannot be applied for load-bearing applications. Scaffolds covered with polymer possessed higher compressive strength and surgical maneuverability, sufficient to withstand the implantation procedures. Notably, in vivo the mechanical properties of scaffolds should increase with time as the tissue grows and the scaffold degrades. Moreover, releasing not only pure (*R*)-3-hydroxybutyric acid but also its oligomers during hydrolytic degradation of the composite material was confirmed, which may be beneficial for the surrounding tissues as a nourishing agent. To further improve ductility and adhesion of P(3HB) film to the scaffold it would be reasonable to try blending P(3HB) with other polymers. This would be the subject of further investigations. Further biological studies considering biocompatibility and antibacterial properties of Ag-βTCP/poly(3hydroxybutyrate) scaffolds are necessary.

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