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Mechanical and biological properties of hydroxyapatite/tricalcium phosphate scaffolds coated with poly(lactic-co-glycolic acid)

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Abstract

Regeneration of bone, cartilage and osteochondral tissues by tissue engineering has attracted intense attention due to its potential advantages over the traditional replacement of tissues with synthetic implants. Nevertheless, there is still a dearth of ideal or suitable scaffolds based on porous biomaterials, and the present study was undertaken to develop and evaluate a useful porous composite scaffold system. Here, hydroxyapatite (HA)/tricalcium phosphate (TCP) scaffolds (average pore size: 500 μm; porosity: 87%) were prepared by a polyurethane foam replica method, followed by modification with infiltration and coating of poly(lactic-co-glycolic acid) (PLGA). The thermal shock resistance of the composite scaffolds was evaluated by measuring the compressive strength before and after quenching or freezing treatment. The porous structure (in terms of pore size, porosity and pore interconnectivity) of the composite scaffolds was examined. The penetration of the bone marrow stromal stem cells into the scaffolds and the attachment of the cells onto the scaffolds were also investigated. It was shown that the PLGA incorporation in the HA/TCP scaffolds significantly increased the compressive strength up to 660 kPa and the residual compressive strength after the freezing treatment decreased to 160 kPa, which was, however, sufficient for the scaffolds to withstand subsequent cell culture procedures and a freeze–drying process. On the other hand, the PLGA coating on the strut surfaces of the scaffolds was rather thin (<5 μm) and apparently porous, maintaining the high open porosity of the HA/TCP scaffolds, resulting in desirable migration and attachment of the bone marrow stromal stem cells, although a thicker PLGA coating would have imparted a higher compressive strength of the PLGA-coated porous HA/TCP composite scaffolds.

Keywords: Hydroxyapatite; Poly(lactic-co-glycolic acid); Porosity; Quenching; Cell attachment

1. Introduction

Bone defects are conventionally treated by replacement with bone grafts and synthetic bone filling materials. However, the tissue engineering approach, which stresses tissue regeneration rather than tissue replacement, has become popular recently. Porous biomaterials (also called scaffolds) used in tissue engineering allow cells to attach, proliferate, differentiate and eventually become specific tissue(s). While scaffolds are expected to disappear after implantation in vivo, a certain level of mechanical strength is required for the scaffolds to withstand a certain level of physiological loading. The open porosity of the porous scaffolds is also important for the tissue's development from cells, where cell culture medium and growth factors can be easily accessed though the open pores. On the basis of previous studies [1–8] on the preparation and characterization of scaffolds for tissue engineering, open porosity, compressive strength and feasibility for cell migration have been realized to be the main criteria for good scaffolds.

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For bone tissue engineering, the design of scaffolds should mimic the structure and properties of the bone extracellular matrices. Because bone consists of a porous composite of interpenetrating phases of hydroxyapatite and collagen, the scaffolds for bone regeneration should be similarly porous composites with interpenetrating ceramic and polymer phases. Porous hydroxyapatite/tricalcium phosphate (HA/TCP) composite was thought to be ideal for the ceramic phase, as it is known for its excellent osteoconductivity and, in some cases, even osteoinductivity [9]. On the other hand, poly(lactic-co-glycolic) acid (PLGA) polymer was selected for the polymer phase, as it is an FDA-approved biodegradable polymer with some degree of ductility and good biocompatibility [10,11]. Thus dual porous HA/TCP/PLGA composite scaffolds should minimize the problems confronted either with the porous sole PLGA polymer (low compressive strength) or with the porous sole HA/TCP ceramics (mechanically weak and brittle). In fact, porous calcium phosphate scaffolds [2,3,5] have been toughened by either polymer coating on the struts or polymer infiltration into the struts (if with open micropores), or both.

Most studies on scaffolds have dealt with the mechanical properties and the cell or tissue ingrowth properties. The thermal shock resistance of ceramic–polymer composite scaffolds has been largely forgotten. The reason for this could be that tissue engineering scaffolds are less likely to be subjected to a temperature higher than body temperature (37 °C), unlike the case of conventional engineering ceramics and composites, where thermal shock resistance is an important thermomechanical property. However, it is justifiable to evaluate the thermal shock resistance of the HA/TCP/PLGA composite scaffolds. Firstly, the porous composite scaffolds are often subjected to a low temperature process, with the lowest temperature being that of liquid nitrogen. For example, when cells are seeded onto the scaffolds and undergo cell culturing, the engineered cells/tissues may need to be stored in a freezer or even in a liquid nitrogen tank. Secondly, one active research area, that of bone-cartilage (osteochondral) tissue engineering, requires the preparation of bilayered composite scaffolds, where a porous polymer scaffold will need to be attached onto a porous ceramic–polymer composite scaffold by a low temperature process, such as the thermally induced phase separation method [6]. Thus, the resistance of the scaffolds to a low temperature thermal shock has been identified as another requirement for tissue engineering scaffolds.

Successful tissue engineering also requires the uniform seeding of cells in scaffolds, and cell seeding should be followed by cell attachment, proliferation and differentiation, and secretion of extracellular matrices. No matter whether a dynamic cell culture, such as a perfusion bioreactor or a conventional static cell culture, is used, well-interconnected pores are prime requirements for the scaffolds. Tissues have often been observed to develop preferentially around the periphery of the scaffolds both in vitro and in vivo, which could be due to the poor circulation of nutrients in the central part of the scaffold or to the poor pore interconnectivity across the scaffold. Thus, it is important to evaluate the cell penetration and the cell attachment on the scaffolds before other processes of the cell to tissue development are studied, and it is regrettable that such studies have not previously been attempted with these calcium phosphate/PLGA composite scaffolds.

2. Materials and methods

2.1. Preparation of composite scaffolds

2.1.1. Coating polyurethane (PU) foams with a ceramic slurry

The ceramic slurry was prepared by mixing 160 g of HA (average particle size 2 μm) with 40 g of β-TCP (average particle size 2.5 μm) using a ball mill under a wet condition for 2 h. The resulting paste was then dried at 100 °C for 24 h followed by heat treatment at 900 °C for 2.5 h, with cooling and heating rates set at 5 °C min^{-1}. The calcined ceramic pieces were milled again for 1 h before 125 ml of distilled water was added. The ceramic–water mixture was further milled for another 24 h. Then 1 ml of 25% ammonium salt of polymethacrylic acid solution (Darvan C, R.T. Vanderbilt) was added to the ceramic paste, followed by mixing for about 30 min. Finally, 1 ml of 2 wt.% polyvinyl alcohol solution was added to produce the final slurry. The PU foams were then dipped into the slurry and gently squeezed several times to allow the slurry to penetrate the foams before the excess slurry was squeezed out. Compressed air from an airgun was used to avoid the blockage of pores. The ceramic slurry-coated PU foams were left to dry at room temperature for at least 24 h.

2.1.2. Sintering of the ceramic-coated PU foams

The ceramic slurry-coated PU foams were fired in an electric furnace (Modutemp Furnace) using a four-stage schedule, comprising (1) heating from room temperature to 600 °C at a rate of 1 °C min^{-1} to burn out the PU foam; (2) raising the temperature from 600 to 1200 °C at a rate of 5 °C min^{-1}; (3) holding the temperature at 1200 °C for 4 h to sinter the ceramic; and (4) cooling the furnace down to room temperature at a rate of 5 °C min^{-1}. The HA/TCP scaffolds were removed from the furnace after it had cooled down. Each sample was weighed and kept in a desiccator.

2.1.3. Coating the sintered HA/TCP scaffolds with PLGA

PLGA pellets (Sigma–Aldrich; PLA:PGA = 75:25; mol. wt = 90,000–126,000) were dissolved in dichloromethane (CH_2Cl_2) solvent, such that every 4 g of PLGA was dissolved in 10 ml of dichloromethane. The sintered HA/TCP scaffolds with small dimensions of about $10 \times 10 \times 10$ mm^3 were then immersed into the PLGA solution for more than 30 s each to allow for complete infiltration. The soaked scaffolds were then placed in a centrifuge

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