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An in vivo evaluation of a novel malleable composite scaffold (polypropylene carbonate/ poly(D-lactic acid) /tricalcium phosphate elastic composites) for bone defect repair

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ABSTRACT

An osteoconductive scaffold can facilitate bone defect repair. In this study, a novel elastic porous composite comprising poly(propylene carbonate) (PPC), poly(D-lactic acid) (PDLA), and β-tricalcium phosphate (β-TCP) was prepared as an osteoconductive scaffold. A salt-leaching method is a non-solvent and easily operated method used to mold up the porous scaffolds. The cylinder scaffold was implanted into a 5 mm in diameter and 10 mm in height rabbit femur condyle defect in 6 rabbits. 4 other rabbits with the same defect that did not have the scaffold implanted served as a control group. Rabbits bone tissue specimens were retrieved at 4 and 12 weeks after surgery. 3 reconstructed and 2 unreconstructed rabbits were examined at each time point. The assessments included a computed tomography (CT) scan and a histological examination. The results demonstrate that a PDT porous scaffold made at a PPC/PDLA/TCP weight ratio of 90/8/2 is (1) biocompatible, yielding a positive cell culture study and minimal inflammatory response in vivo; (2) malleable, such that the scaffold can be molded into the bone defect easily without fracturing; and (3) biodegradable and osteoconductive, promoting the progressive formation of new bone into the bone defect. These results indicated that combination of this scaffold with osteoinductive agents such as bone morphogenetic protein, demineralized bone matrix, or mesenchymal cells may generate new biomaterial for bone defect repair.

1. Introduction

Osteoconductive materials are expected to facilitate the repair of bone defects by acting, preferably temporarily, as a scaffold for growing capillaries and osteoprogenitor cells [1]. The most widely investigated osteoconductive compounds are made with hydroxyapatite (HA) [2]. Biphasic calcium phosphate made of HA and β-tricalcium phosphate has been used clinically to fill long bone defects. The major drawback of HA pertains to its physical properties [3]. The block form of the substance is brittle and difficult to shape, whereas the granular form does not appear to demonstrate sufficient structural stability and is difficult to contain within the area of reconstruction. Although the cement form is easily shaped to fit a given bone defect, because of its microporous nature (<5 μm), minimal bony or vascular ingrowth occurs, and the cement remains largely avascular [4]. To address these issues with using HA, we developed a novel, malleable, biodegradeable, and osteoconductive type of porous scaffold comprising poly propylene carbonate (PPC), poly D-lactic acid (PDLA), and β-tricalcium phosphate (β-TCP). The resulting scaffolds were referred to as PPC/PDLA/β-TCP (PDT) scaffolds.

PPC is a new type of biodegradable polymer that was developed by copolymerizing propylene oxide and carbon dioxide. PPC has attracted increasing interest in both research and industry contexts because the use of PPC in place of alternative polymers could reduce greenhouse gas emissions and conserve scarce fossil-based raw materials [5,6]. PPC is potentially suitable for applications in the medical field because of its excellent biocompatibility, high biodegradability, and low toxicity [7].

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PDLA, which can be made from renewable resources, has elicited widespread interest for decades because of its remarkable biodegradability and biocompatibility. However, the properties of PLA-based materials have to be tailored so that all these advanced applications can be realized. For example, in tissue engineering applications, a successful PLA-based scaffold must provide the required porosity, the specified size and shape of the object, and the required physical properties, such as extensibility and strength to promote tissue regeneration and guide tissue growth [8–11]. All these drawbacks significantly limit the application of PLA-based materials. Much effort has been devoted to developing new PLA-based materials with required properties suitable for biomedical applications.

It is well-known that blending PLA polymer with other polymers possesses a cost-effective way to obtain polymer blends with desired properties. PLA can be blended with flexible polymers such as poly(ε-caprolactone) (PCL), poly(butylene succinate) (PBS), and poly(butylene succinate adipate) (PBSA) to achieve improve elongation and flexibility [12]. Also, the resulting PLA blends retain the biodegradability since these polymers are biodegradable.

However, the issues of immiscibility and phase separation behavior of polymer blends have to be addressed. Therefore, poly(propylene carbonate) (PPC) is a suitable choice to be incorporated with PLA to obtain the desired properties of PPC/PLA blends since they have similar chemical structures and consequently expected to be miscible and compatible to some extent [13, 14]. The PLA polymer possesses good mechanical properties but poor elasticity, while PPC exhibits good elasticity but poor mechanical strength. The PPC in the PLA-rich blends can toughen the PLA polymer, while the PLA in the PPC-rich blends is expected to reinforce the PPC polymer. It is found that the phase structure of PLA/PPC blends depends strongly on the blend ratio, heat-treatment temperature, and the heat-treatment time [15].

Bioceramics are one of the most widely used and studied class of materials that interface with bone for clinical application as a filler in dentistry and orthopedic for decades [16]. Among these bioceramics, calcium phosphates (CaPs), including hydroxyapatite (HA), with the chemical composition $Ca_5(PO_4)_3OH$, β-tricalcium phosphate (β-TCP), with the chemical composition $Ca_3(PO_4)_2$, and biphasic calcium phosphate (BCP), which refers to composites of HA and β-TCP, have received most attention. Incorporating these osteoconductive CaP fillers into the PLA matrix has been proved to improve mechanical strengths of the resulting composite so that it can be used in the load-bearing areas for implants. The key factors for optimizing the strength include the inherent characteristics of the filler (crystallinity, size, shape) and its volume fraction, dispersion of the fillers within the matrix, integrity of the interface, and the interfacial bond strength [17].

In our previous study, we confirmed the osteoconduction of a PPC scaffold tested with osteoblast cultures in vitro [18]. In the present study, we evaluated the potential for using a PPC scaffold for bone regeneration in vivo. More specifically, we evaluated bone regeneration rates in a rabbit condyle model implanted with the scaffolds used were the aforementioned PDT scaffolds. The PDT scaffolds were designed to be malleable according to the different ratios of tough PDLA and soft PPC used in their composition. Relatedly, the PPC effectively provides the elastic properties of the given scaffold, while the PDLA ensures that the scaffold has suitable strength. Therefore, a biocompatible and biodegradable PPC was used to adjust the bending strength and bending modulus of the PDT composites. Moreover, a bioceramic material, β-TCP, which has been commercialized as a biodegradable bone substitute for use in orthopedic and dental applications, was also introduced to further optimize the mechanical and biological properties of the PPC composites. The purpose of this study was to evaluate the tissue compatibility, biodegradeation, and osteoconductivity of the different PDT scaffolds at up to 12 weeks after implantation in rabbit femur bone defects.

2. Materials and methods

2.1. Preparation of PDT elastic porous scaffolds

Porous PDT scaffolds were prepared using the salt-leaching method. For each scaffold, a mixture of medical-grade β-TCP (Sigma–Aldrich, Missouri, USA; particle size: 2–8 μm), medical-grade PPC (Sigma–Aldrich, Missouri, USA; molecular weight: 50,000 Dalton), and PDLA (Bio-Invigor Corporation, Taipei, Taiwan; inherent viscosity: 0.55 dL/g) was prepared [19]. Subsequently, 8 g of sodium chloride (NaCl; diameter: 104–250 μm) was added to 2 g of the PDT polymer and mixed thoroughly. Regarding the weight ratio of the polymer mixture, the weight ratio of the NaCl to each verified PDT composite was fixed at 2:8. Each The PDT composite with NaCl was then molded with a hot press for 3 h at 50 °C and at a pressure of 13.79 MPa [20]. The PDT composite with NaCl was then immersed into deionized water for 72 h to leach out the NaCl. The deionized water was changed every 4 h The structural images of the resulting PDT porous composites were obtained using a scanning electron microscope (SEM, Hitachi S-3000H, Tokyo, Japan). The void density of the resulting porous PDT scaffolds was analyzed using Scion Image software (Scion Corporation, N.Y., USA).

2.2. Evaluation of biocompatibility

To evaluate the biocompatibility of each PDT scaffold, an in vitro test was performed in accordance with the ISO 10993-5:2009(E) guidelines by using the L929 mouse fibroblast cell line. Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% (v/v) fetal bovine serum (Gibco) was used as a culture medium. For an indirect contact assay, triplicates for each PDT scaffold were placed on 6-well plates (Falcon®, BD Biosciences) containing 2 ml of the culture medium and stored in an incubator (37 °C, 5% CO_2, fully humidified) for 72 h in preparation for material extraction. The liquid extracts were then used to cultivate L929 fibroblasts, and L929 cells were seeded in 24-well plates (Falcon®, BD Biosciences) at an initial density of 5×104 cells/cm^2 for 24 h. The cell proliferation rate of the material extraction was measured using a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. The absorbance was measured at 570 nm.

2.3. Surgical procedures

The animal study was approved by the Laboratory Animal Center of the National Defense Medical Center, Taipei, Taiwan (Approval No. IACUC-14-137). Surgical procedures were performed under general anesthesia administered through an intramuscular injection of a Zoletil 50 and 2% Rompun solution (1:2 ratio, 1 ml/kg). Ten adult male New Zealand white rabbits weighing 2.5–3.0 kg were used. The surgical protocol was adapted from a previous study [21]. The PDT scaffolds (PPC: PDLA:<beta>-TCP= 90:8:2 weight ratio) were selected to serve as implants for the rabbit femur defect model based on their excellent elastic properties with better tensile and bending mechanical properties (Fig. 1). In each rabbit, the femur bone was exposed and a cylindrical bone defect with a diameter of 5 mm and a depth of 10 mm was created using a drilling burr attached to a slow-speed dental hand piece (Implant engine X-cube; SAESHEN, South Korea). The bone defect was then reconstructed with PDT and phosphate-buffered saline (PBS) in 6 of the rabbits, whereas it was left unreconstructed in 4 of the rabbits (Fig. 2). For each rabbit, the periosteum was then closed with 5–0 Vicryl sutures, and the skin was closed with 4–0 nylon

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