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Preparation of a hemiporous hydroxyapatite scaffold and evaluation as a cell-mediated bone substitute

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Abstract

A hemiporous hydroxyapatite (HAp) scaffold was prepared to support the tissue engineered approach to the restoration of damaged bone. The scaffold comprised a porous cell-seeded part and a non-porous load bearing part. A wet processing technique of HAp suspensions was used to shape the hemiporous body. The structure of the porous part was tailored using a stack of heat treated porogen placed on the plaster. The prepared specimen had approximately 30 layers of connected pores, which could accommodate sufficient human bone marrow stromal cells (hBMSCs). The result of an *in vitro* test showed that hBMSCs successfully proliferated and produced extracellular matrices even at the pore in the deep portion of the scaffolds. The *in vivo* test in the distal femur of a rabbit showed the formation of new fibrous tissue and tubular vessels with red blood cells in the hBMSCs-seeded scaffold from the pores at the deepest portion as well as from the pore at the periphery of the scaffold. The result was in distinct contrast with the scaffold without cell loading. The preloading of cell was thus very effective in the migration of cells in spite of the unconfirmed connectivity among pores. The present casting approach had the merits of simplicity and versatility in tailoring the scaffold structure without an elaborate device.

Keywords: Hydroxyapatite; Bone marrow stromal cell; Scaffold; Preloading; *In vivo*

1. Introduction

Many challenges are involved in the treatment of bone loss conditions such as non-union, periprosthetic bone defect, and posttraumatic or postsurgical bone loss. In many cases, adjunctive measures such as bone-grafting or bone substitute implantation are required to stimulate bone-healing and fill bone defects. Autologous or allogenic bone graft, demineralized bone matrix, and synthetic bone substitute are options for these conditions. However, their shortcomings include donor-site morbidity, limited volume available, immunogenicity, disease transmission risk, and high costs [1,2]. Especially, synthetic bone substitutes do not have internal osteoinductive acting cells, rendering their biological functions limited [1,2].

Cell-seeded bone substitute is a novel tissue engineered approach used to render osteoinductive synthetic bone substitute. Before implantation, cells are infiltrated into the substitute and are then cultured. Thus, the scaffold inevitably requires a highly open structure for the accommodation of sufficient cells. To ensure the connected structure of the pores, the porosity of the substitute needs to be fairly high. However, the increased porosity is critically disadvantageous to the load bearing capability, *i.e.* strength. The decrease in strength limits an applicability of the scaffold as well as the ease of handling during cellular introduction and implantation. It should be noted that the strength of natural bone relies on the dense cortical bone. Therefore, inspired from the natural structure of bone, the strengthened scaffold can be designed by providing a dense support for load bearing.

In this context, the Hydroxyapatite (HAp) specimen composed of porous body and dense support is prepared and evaluated. The specimen was termed as hemiporous scaffold, since half of the scaffold was porous. HAp, the mineral part of natural bones, is capable of guiding bone formation [3]. In a previous report [4], even chemical bonding with natural bone was claimed due to the

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chemical similarity. HAp has, therefore, been widely used as a synthetic bone substitute [5–7].

Human bone marrow stromal cells (hBMSCs) are potentially good candidates for the cell-mediated tissue engineered approach to address a variety of unsolved medical situations, particularly in the musculoskeletal area, owing to their relatively good availability and biological characteristics [8–10]. hBMSCs have the capacity of self-renewal and can differentiate into several types of mesenchymal cells, including osteoblasts, chondrocytes, and adipocytes [8–10].

In this study, a hemiporous HAp scaffold was evaluated both *in vitro* and *in vivo* with hBMSCs. The hBMSCs were cultured in the scaffold for 14 days before implantation to achieve migration and successive proliferation. In addition, we evaluated the tissue regeneration ability of hBMSCs-seeded hemiporous HAp in the distal femoral bone defects of rabbits. The results were compared with the same scaffold without cell loading. We hypothesized that the cellular responses of hBMSCs in the porous region of a hemiporous HAp scaffold might be acceptable *in vitro*. We also postulated that the hBMSC-loaded hemiporous HAp scaffold would enhance bone formation at an orthopedic surgical site *in vivo*.

2. Materials and methods

2.1. HAp suspension preparation

An aqueous processing route was selected for the preparation of the porous scaffold. HAp powder (Sigma-Aldrich, St. Louis, MO, USA; 34.0–40.0% Ca) was attrition milled (KMC-1B, KMC, Seoul, Korea) using 5 mm diameter zirconia balls as media. Darvan C (Vanderbilt Co. Norwalk. CT, USA) was introduced as a dispersant. The suspension was prepared through the measurement of the viscosity as a function of various parameters such as pH and solid loading. The pH of the suspension was controlled by introducing NH_4OH. A concentrated HAp suspension with minimized viscosity was prepared through the careful control of parameters including the amount of dispersant, pH, solid loading and milling time as reported previously [11]. The particle size in the suspension was estimated to be 0.3 μm according to the analysis based on laser diffraction (Mastersizer 2000, Malvern, England).

2.2. HAp substitute with porous layer on the surface

Porogen Sephadex G-50 polymer beads (Sigma-Aldrich, St. Louis, MO. USA, Fig. 1a) with a diameter of 300–350 μm provided the template to form a porous structure. Sephadex is a cross-linked dextran gel synthetically derived from the polysaccharide, dextran. Complete removal of porogens could be verified by burning out at 400 °C. The stack of polymer beads with uniform thickness was prepared by placing them between two glass plates. The stack was heat treated at 230 °C for 2 h to partially bond the porogens (Fig. 1b). The heat treated stack was placed on a plaster block with a cylindrical rubber mold in a similar way to that reported [12] previously. The green body was formed by pouring the HAp suspension into the cylindrical mold. With the drying of the slip cast specimen, shrinkage of the green body was observed. The specimen was thus dried at room temperature to facilitate slow drying. After drying, the specimen was heated to 400 °C at a rate of 2 °C/min to burn out the porogens and was subsequently consolidated by sintering at 1200 °C for 2 h.

2.3. Structural characterization

The specimen was impregnated in the diluted resin to evaluate the connectivity of the pores. As soon as the resin solidified, the specimens were vertically bisected with a diamond saw for microstructural analysis. The exposed surface was sequentially polished with SiC abrasive paper to reveal the pores filled with resin. Scanning electron microscopy was performed using a JSM-6300 apparatus (Jeol, Tokyo, Japan) at an accelerating voltage of 20 kV. Connectivity was estimated by measuring the volume fraction of resin-filled pores among the total volume of pores from the polished cross section.

2.4. Measurement of load bearing capability

The load bearing capability of the specimen was measured using a model 810 universal testing machine (MTS810, Eden Prairie, MN, USA) at a crosshead speed of 0.5 mm/min. The load for fracture was tested either by axial or diametral compression.

2.5. Isolation and seeding of hBMSCs

After institutional review board approval (IRB H-1010-074-337), fresh bone marrow was aspirated from the iliac crests of patients undergoing total hip arthroplasty. A 14-gauge needle was inserted into the iliac tuberosity adjacent to the anterior superior iliac spine, and 6–7 mL of marrow was aspirated. Two more aspirates were obtained through the same cortical hole, but at a different depth. Twenty milliliters of bone marrow aspirates were collected from each patient into a syringe containing 6000 units of heparin. hBMSCs were isolated from the marrow aspirates using a modified version of the previously detailed procedures [10,13]. The aspirates were washed with Dulbecco's phosphate-buffered saline (DPBS; GibcoBRL®, Gaithersburg, MD, USA), and the cells were recovered following 10 min of centrifugation at 900*g*; this process was repeated once. The washed cells were subsequently re-suspended in DPBS to a final density of 4×10^7 cells/mL. A 5 mL aliquot was layered over 1.073 g/mL of Percoll solution (Pharmacia®, Piscataway, NJ, USA) in a 50 mL conical tube and centrifuged for 30 min at 1100*g*. Nucleated cells collected at the interface were recovered and counted using a hemacytometer. The collected nucleated cells were then re-suspended in a human mesenchymal stem cell (MSC) medium and plated in 150 mm diameter Petri dishes at a density of 2×10^6 cells/cm^2 in 30 mL of medium. The human MSC medium consisted of Dulbecco's Modified Eagles Medium-Low Glucose (DMEM-LG; GibcoBRL®) supplemented with 10% fetal bovine serum (FBS; GibcoBRL®)