

Reconstruction of goat femur segmental defects using triphasic ceramic-coated hydroxyapatite in combination with autologous cells and platelet-rich plasma

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Abstract

Segmental bone defects resulting from trauma or pathology represent a common and significant clinical problem. In this study, a triphasic ceramic (calcium silicate, hydroxyapatite and tricalcium phosphate)-coated hydroxyapatite (HASi) having the benefits of both HA (osteointegration, osteoconduction) and silica (degradation) was used as a bone substitute for the repair of segmental defect (2 cm) created in a goat femur model. Three experimental goat femur implant groups – (a) bare HASi, (b) osteogenic-induced goat bone marrow-derived mesenchymal stem cells cultured HASi (HASi + C) and (c) osteogenic-induced goat bone marrow-derived mesenchymal stem cells cultured HASi + platelet-rich plasma (HASi + CP) – were designed and efficacy performance in the healing of the defect was evaluated. In all the groups, the material united with host bone without any inflammation and an osseous callus formed around the implant. This reflects the osteoconductivity of HASi where the cells have migrated from the cut ends of host bone. The most observable difference between the groups appeared in the mid region of the defect. In bare HASi groups, numerous osteoblast-like cells could be seen together with a portion of material. However, in HASi + C and HASi + CP, about 60–70% of that area was occupied by woven bone, in line with material degradation. The interconnected porous nature (50–500 μm), together with the chemical composition of the HASi, facilitated the degradation of HASi, thereby opening up void spaces for cellular ingrowth and bone regeneration. The combination of HASi with cells and PRP was an added advantage that could promote the expression of many osteoinductive proteins, leading to faster bone regeneration and material degradation. Based on these results, we conclude that bare HASi can aid in bone regeneration but, with the combination of cells and PRP, the sequence of healing events are much faster in large segmental bone defects in weight-bearing areas in goats. © 2009 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

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1. Introduction

Segmental bone defects resulting from trauma or pathology represents common and significant clinical problem. Osteotomy followed by bone distraction (the Ilizarov technique) is the principal treatment option for these defects as they typically do not heal spontaneously [1]. However, the high rate of success by this technique

is counterbalanced by pin tract infection, the patient's uncomforness and a long recovery time [2]. The usefulness of autogenous bone grafting is demonstrated by the long-standing history of orthopaedic practice as autogenous bone grafts possess inherent osteoconductivity, osteogenicity and osteoinductivity. Nevertheless, there is often a limited supply of suitable bone and its collection is frequently associated with donor site morbidity [3]. Unlike autologous bone, allogeneous grafts are widely available and do not require any additional surgery in the patient. However, allogeneous bone needs to undergo processing techniques, such as lyophilization, irradiation

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or freeze-drying, to avoid any risk of provoking an immunogenic reaction [4].

The synthetic bone graft, which mimics the mineral composition of bone such as hydroxyapatite (HA), has fulfilled many of the properties of an “ideal” bone substitute. However, HA is resistant to degradation in vivo, which occurs at a rate of 1–2% per year [5]. Currently, a number of silicon-containing bioactive materials are of great interest because of their ability to nucleate the precipitation and formation of a calcium phosphate layer on the implant surface and thus enhance bone formation. In addition, silica-based bioactive ceramics can undergo degradation in line with bone formation [6,7].

In a recent study, we evaluated the physicochemical characteristics and in vitro cytocompatibility behaviour of a triphasic ceramic-coated HA (HASi) using goat bone marrow-derived mesenchymal stem cells [8]. For synthesizing HASi, HA blocks were prepared and dipped in silica sol with the hypothesis that silicon ions will incorporate into HA as an outer coating layer, while the main core of HASi remains as HA. Thus the goal was to utilize the dual properties of HA (osteoconduction, osteointegration) and silica (osteoconduction, osteointegration and degradation) in one product. The material has an interconnected porosity (67% porosity) and the pore size ranges between 50 and 500 μm . The major (core) phase of HASi is HA, while the outer coating layer contains peaks for HA ($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$), β -tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) and calcium silicate (Ca_2SiO_4) [8,9]. The core of HASi is polycrystalline and has clear grain boundary characteristics, while the outer coating layer is porous, less crystalline and has no clear grain and grain boundary. This is due to the formation of a Si-rich liquid phase, which penetrates to a depth of about 1 mm.

In large bone defects, which are unable to heal spontaneously, it is important to restore the structural integrity and its function within the shortest possible period. The optimization of such a process relies on the interplay between two interdependent elements other than the bone substitute: (i) progenitor cells for osteogenesis and (ii) growth factors for osteoinduction [10]. Bone-marrow-derived mesenchymal stem cells (BMSCs) are a very attractive source for regenerative medicine due to their ability to differentiate to several cell lineages (adipocytes, chondrocytes and osteoblasts) and promote bone-specific protein synthesis and mineralization in an osteoconductive environment [11,12].

In terms of osteoinductive growth factors, platelet-rich plasma (PRP) has been in use in much clinical and experimental bone reconstructive surgery [13]. The activated platelets are a source of growth factors, such as platelet-derived growth factor, transforming growth factor β , fibroblast growth factor, vascular endothelial growth factor (VEGF) and insulin-like growth factor [14]. For this reason, the platelet concentrate delivered straight to the site of injury attracts and activates several functions of cells

(such as bone cells and periosteum cells, as well as osteoclasts and fibroblasts) in the surrounding tissues [15].

Herein we focused on the progress of bone regeneration and material degradation in the goat (g) femur segmental defect for a period of 2 months. The experimental animals were divided into three groups: (i) bare HASi (HASi); (ii) osteogenic-induced gBMSCs cultured HASi (HASi + C); and (iii) osteogenic-induced gBMSCs cultured HASi together with the delivery of PRP at the defect site (HASi + CP).

2. Materials and methods

2.1. Material preparation

Hydroxyapatite powder was synthesized by a wet precipitation method involving calcium nitrate and ammonium dihydrogen phosphate in the stoichiometric proportion at a pH of 11 and a temperature of 80 °C. The precipitated HA powder was freeze-dried and washed with distilled water to remove any surface impurities, such as nitrate and ammonium ions. HA powder with particle size of <125 μm was mixed with aqueous solution of polyvinyl alcohol and glutaraldehyde solution and stirred for 30 min. Benzoyl peroxide dispersed in benzene and *N,N*-dimethyl aniline were added to the resulting frothy slurry, which was stirred to mix thoroughly. The resulting frothy and viscous slurry was poured into plastic moulds and allowed to dry at room temperature. After drying, the blocks were biscuit fired at 300 °C for 1 h to remove the binder and then sintered at a temperature between 1100 and 1300 °C for 1 h to obtain porous ceramics. HA blocks prepared by the above process were dipped in silica sol prepared by the hydrolysis of tetraethyl orthosilicate in an ethanol–water system for 1 min and sintered at 1200 °C for 2 h to obtain a coating over HA. The materials were polished to form a hollow cylinder with outer and inner diameters of 2 cm and 7 mm, respectively, and a length of 2 cm. Prior to cell seeding, the material were steam sterilized and conditioned by incubating in α -minimal essential medium (α -MEM) with 10% foetal bovine serum (FBS) at 37 °C for 24 h.

2.2. Cell culture and osteogenic induction

Goat bone marrow aspiration was conducted [8] as per the guidelines of the Institutional Animal Ethics Committee (IAEC) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Bone marrow was aspirated from four goats in HASi + C and HASi + CP groups in order to use autologous cells for transplantation. The isolated gBMSCs were cultured in α -MEM containing 10% FBS, 100 U ml^{-1} penicillin and 100 $\mu\text{g ml}^{-1}$ streptomycin (Gibco, India). For osteogenic induction, the cells were cultured in α -MEM supplemented with 15% FBS, 10 mM β -glycerophosphate,

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