Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 4 (2008) 173–181
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

A triphasic ceramic-coated porous hydroxyapatite for tissue engineering application

Manitha B. Nair, S. Suresh Babu, H.K. Varma, Annie John *

Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Poojappura, Trivandrum 695 012, India

Received 2 January 2007; received in revised form 25 June 2007; accepted 9 July 2007
Available online 27 July 2007

Abstract

Scaffolds which encourage the incorporation of a cell source for tissue engineering applications are critical determinants for clinical defects. Over the years, a number of biomaterials have emerged for cell support and growth, but only a few have demonstrated clinical efficacy. We therefore investigated an in-house-developed silica-based bioactive ceramic for its ability to support and sustain the growth of bone marrow-derived mesenchymal stem cells (BMSCs) in vitro. For this, MSCs aspirated from goat bone marrow were isolated and culture expanded on a novel triphasic ceramic composite coated hydroxyapatite (HASi) scaffold comprising hydroxyapatite, tricalcium phosphate and calcium silicate. The viability of cells that harbored on and within the material was ensured through fluorescence-activated cell sorting and confocal laser scanning microscope and for their anchorage sites by scanning electron microscopy. Interestingly, over the days in culture, cell–cell interactions gradually morphed into woven cell-sheets that spanned across the surface of the HASi, forming a canopy. To conclude, we have attempted to carry out the preliminary cytocompatibility studies of this novel ceramic to establish its appropriateness for bone tissue engineering application which is an important criterion in orthopaedic transplantation and regenerative surgery.

Keywords: Mesenchymal stem cells; Bioactive ceramic; Cell sheet; Tissue-engineered construct; Bone tissue engineering

1. Introduction

Clinical strategies in the field of regeneration include autografts and allografts, both of which have inherent limitations, such as limited supply, increased morbidity and disease transmission potential [1]. Tissue engineering is a new concept that aims at developing biological substitutes by incorporating cells or growth factors into a three-dimensional scaffold to mimic native tissue architecture and function [2]. A common approach is to isolate specific cells through a small biopsy from a patient and then permit them to grow on a scaffold under controlled conditions. This limits the need for the migration and differentiation of indigenous cells within defect sites and in turn accelerates tissue regeneration [3]. However, this methodology required for the isolation of differentiated cells is time consuming and has limited expansion potential. To overcome this problem, bone marrow-derived mesenchymal stem cells (BMSCs) can be used and easily isolated, and have high expansion and differentiation potential [4]. Several culture techniques have been developed and these purified, culture-expanded BMSCs are capable of differentiating along the osteogenic, chondrogenic, adipogenic and marrow stromal lineages [5,6], depending on the conditions of the external milieu in which they are exposed, thereby manipulating the fate of the cells.

The challenge facing the tissue engineering field is that the physico-chemical nature of the material surface and the biological behaviour of the cell should coordinate in a sequestered pattern to form a functional tissue. So, the selection of the correct biomaterial is important, with properties such as pore size, pore structure, surface topography,

* Corresponding author. Tel.: +91 471 2520215; fax: +91 471 2341814.
E-mail addresses: karippacheril@yahoo.com, ajkari@sctimst.ac.in (A. John).

1742-7061/$ - see front matter
doi:10.1016/j.actbio.2007.07.004

chemical composition, hydrophilicity, surface energy and charge and degradation [7] crucial and relevant. In orthopaedic applications, a range of bioactive ceramics such as hydroxyapatite (HA), tricalcium phosphate (TCP), bioglass and glass ceramics have been employed because of their similarity in composition to the mineral phase of natural bone and excellent bone bonding ability [8,9]. Although HA exhibits good osteoconductive and osteointegrative properties [10], success is impaired by its poor resorption properties [11]. Several contemporary comparative studies have demonstrated the efficacy of silica-based bioactive ceramic products as better biodegradable material than HA. This is because silica acts as a nucleating apatite for bone formation and thereby promotes and enhances bone bonding in vivo [12]. In addition, the surface chemistry of silica-based materials is amenable to extracellular matrix proteins for the formation of a strong protein film on the material which facilitates cell adhesion and other cellular activities. So, in order to improve the criteria required for successful bone grafting, the logical approach is to utilize the properties of more than one material to combine the strength of the parent phases and simultaneously to minimize any undesirable characteristics. In this study a trial has been attempted to utilize the dual benefits of HA and SiO in one product by coating hydroxyapatite with silica sol to develop a novel porous bioactive ceramic, HASi, composed of hydroxyapatite (HA), tricalcium phosphate (TCP) and calcium silicate. A tissue-engineered construct was thereafter fabricated in vitro using HASi and goat bone marrow-derived mesenchymal stem cells (gBMSCs) and analysed for its cell viability, proliferation and morphology in order to explore its application for large bone defects in skeletal reconstruction.

2. Materials and methods

An in-house-prepared HASi scaffold was used for the study.

2.1. Material preparation

Hydroxyapatite (HA) powder was synthesized by a wet precipitation method involving calcium nitrate and ammonium dihydrogen phosphate in stoichiometric proportions at a pH of 11 and a temperature of 80 °C [13]. The precipitated HA powder was freeze-dried and washed with distilled water to get rid of surface impurities such as nitrate and ammonium ions. HA powder having particle size less than 12 μm was mixed with aqueous solution of polyvinyl alcohol and gluteraldehyde solution, and stirred for 30 min. To the resulting frothy slurry, benzoyl peroxide dispersed in benzene and *N*,*N*-dimethyl aniline were added and 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 produce porous ceramics. The hydroxyapatite blocks thus produced were dipped in a silica sol prepared by the hydrolysis of tetraethyl orthosilicate (TEOS) in an ethanol–water system for 1 min. The resultant coated HA ceramic was sintered at 1200 °C for 2 h to get a coating over hydroxyapatite. It was later polished to get a size of 5 mm diameter and 5 mm thickness, and subjected to ultrasonic cleaning for the complete removal of fine powders adhered over the surface. Prior to cell seeding, the material was steam sterilized and conditioned by placing in α-MEM and incubated at 37 °C for 24 h.

2.2. Material characterization

2.2.1. Porosity

Porosity studies were done by mercury intrusion technique (Quantachrome, Pore Master®33). A few discs of known weight were loaded in the penetrometer cell of the equipment and evacuated, and then filled with mercury under pressure. The volume of the mercury that intruded was measured as a function of pressure. The bulk density of the sample was calculated from the difference in weight between an empty cell filled with Hg and a cell with the sample filled with Hg. The total porosity and the percentage of the open porosity were also evaluated from the total volume of the Hg intruded.

2.2.2. Scanning electron microscopy and energy-dispersive X-ray analysis

The HA and HASi blocks were gold coated in an ion sputter (Hitachi E101) and their microstructures examined by scanning electron microscopy (SEM) (Hitachi S2400). The element present in the material was plotted through energy-dispersive X-ray analysis (EDAX; OXFORD X-ray microanalysis software).

2.2.3. X-ray diffraction

The phase analysis and crystallinity check-up of HASi were done by an X-ray powder diffraction technique. For this, a 10 mm HA disc was prepared, coated with silica sol and sintered as per above schedule. The sintered pellet was scanned between $2\theta = 20$ and 40° at a rate of 2° min^{-1} with a step size of 0.02° using Cu Kα1 radiation at a voltage of 40 kV and a current strength of 30 mA (Siemens D-5005). The coating layer of HASi pellet was removed by grinding and thereafter scanned for analysis of the inner core.

2.2.4. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) analysis was conducted on a Thermo Nicolet 5700 spectrometer and the spectra were collected in the diffuse reflectance (DRIFT) mode. Samples were prepared by mixing the sample powder with optical grade KBr powder; pure KBr was used as the background. The spectra were recorded at a resolution of 4 cm^{-1} and scanned between 400 and 4000 wave number cm^{-1}. The average number of scans was 200.

ID	Title	Pages
1983	A triphasic ceramic-coated porous hydroxyapatite for tissue engineering application	9