Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Biomolecular Engineering 24 (2007) 75–80
Biomolecular Engineering
www.elsevier.com/locate/geneanabioeng

Fabrication of a three-dimensional nanostructured biomaterial for tissue engineering of bone

E. Garreta [a], D. Gasset [a], C. Semino [a,b], S. Borrós [a,*]

[a] Barcelona Bioengineering Center, Institut Químic de Sarrià, Universitat Ramon Llull, Via Augusta 390, 08017 Barcelona, Spain
[b] Center for Biomedical Engineering, Massachussets Institute of Technology, 500 Technology Square, NE47-383, Cambridge, MA 02139, USA

Abstract

A plasma process for the surface modification of HA powders has been developed. Acrylic acid and acrylic acid/octadiene plasma deposited films onto HA particles have demonstrated to interact with SBF allowing the calcium dissolution–precipitation mechanism. Therefore, a nanostructured composite between HA and a self-assembling peptide scaffold (RAD16-I) has been developed. The differentiation of mESC in this scaffold has been studied, in order to test the osteogenic capacity of the new composite material. We have observed that the mESC can be iduced to produce Ca salts (mineralization) in a 3D-microenvironment and moreover, this activity can be enhanced by the presence of HA particules into the nanofiber scaffold.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Cartilage; Hydroxyapatite; Bone tissue engineering; Nanostructured composite

1. Introduction

The knowledge of articular cartilage, calcified cartilage and bone tissue architectures as well as the biological mechanisms involved in the maintenance of their structure and function is essential for the design of new biomaterial scaffolds with properties such as tissue compatibility, regeneration induction and growth that could potentially enhance inate restorative capacity. For instance, bone and cartilage, like any other tissue, present a complex hierarchical organization with a unique characteristic: an interface zone between both tissues, consisting of an intimate transition of one tissue type into the other, essentially important for the proper biomechanical function of this unique anatomical structure. Thus, collagen fibers, non-collagenous proteins and proteoglycans – which compose the organic bone and cartilage matrix – are arranged in a very specific manner where appropriate mineral deposition is required in order to fulfill their mechanical functions (Zizak et al., 2003; Rho et al., 1998; Boskey et al., 1992; Landis et al., 1995). Recently, Zizak et al. have characterized the nanometer-sized mineral particles in the transition zone from bone to cartilage. These mineral particles are composed of hydroxyapatite (a crystalline form of calcium phosphate) with small but significant amounts of impurities such as HPO_4, Na, Mg, citrate, carbonate, K and others. It has been proved that the mineral particle thickness parameter is very similar for bone and mineralized cartilage (from 2.0 to 4.2 nm), indicating that the determination of particle size is not only governed by the collagenous matrix structure but also by the non-collagenous proteins. In addition, mineral particles in calcified cartilage are preferably oriented perpendicular to the interface while in bone they are oriented parallel to it, perhaps reflecting the morphology of the underlying organic matrix (Zizak et al., 2003).

It has been widely described the ability of bioceramics such as hydroxyapatite (HA) and bioactive glass ceramics to form a bonding with the surrounding bone tissue (Hench and Wilson, 1993; Jarcho, 1986). However, these materials alone do not match the specific requirements of bone tissue and specially the calcified cartilage interface. For this reason, researchers try to combine these inorganic materials with organic matrices that can improve cell-biomaterial scaffold interactions thereby enhancing new tissue growth. In this sense, a new nanostructured composite formed of HA and a synthetic self-assembling peptide was developed in this work. HA was synthesized by the sol–gel method presented using calcium nitrate and triethyl phosphite as precursors. The self-assembling peptide (obtained by by solid-phase synthesis) consisted of an alternating sequence of hydrophilic–hydrophobic aminoacids, specifically the AcN-$(RADA)_4$-$CONH_2$ sequence (RAD16-I). The hydrophilic

* Corresponding author. Tel.: +34 93 589 04 13; fax: +34 93 205 62 66.
E-mail addresses: semino@mit.edu (C. Semino), s.borros@iqs.url.edu (S. Borrós).

1389-0344/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioeng.2006.05.017

sequence presents alternating positively charged and negatively charged side chains. This molecule in aqueous solution adopts a β-strand secondary structure with the hydrophilic side chains in one side and the hydrophobic side chains on the other side. A self-assembling process forms a β-sheet tape that develops into a nanofiber of several microns length. This synthetic peptide has been used before to culture different cell types (Semino et al., 2003, 2004; Genove et al., 2005) and one of its main advantatges is that it forms a three-dimensional structure composed of nanofibers which mimics very well the biological three-dimensional environment of cells, allowing growth, migration and differentiation. In addition, a plasma polymerization technique was used to modify the surface of the HA particles in order to achieve an appropriate compatibility of them with the peptide as well as a good homogeneity of the final composite. In general, plasma polymerization processes allow deposition of thin films onto substrates of complex geometry by reaction of gas-phase species, giving rise to a uniform distribution of functional groups (Whittle et al., 2002; Chu et al., 2002). A wide range of compounds can be chosen as a monomer for plasma polymerization, providing a great diversity of possible surface modifications. In this work we have tried to modify the hidroxyapatite powder with acrylic acid in order to increase its compatibility with the self-assembly peptide and obtaining a self-dispersing hidroxyapatite in water.

Therefore, the objective of this work was to develop a composite consisted of hydroxyapatite, modified by the plasma polymerization technique, and a self-assembling peptide scaffold for its application in calcified cartilage interface and bone tissue engineering. The evaluation of the *in vitro* bioactivity of the composite prepared by mixing the two components in different proportions has been tested, by inducing mouse embryonic stem cells (mESC) to undergo osteogenic differentiation inside this synthetic nanostructured scaffold.

2. Materials and methods

2.1. Development of the hydroxyapatite/self-assembling peptide composite

2.1.1. Surface modification of HA particles by plasma polymerization

The plasma deposition apparatus consists of a tubular glass vessel composed of a glass cylinder of 6 cm-diameter/50 cm-length and two round vessels coupled to each end of the cylinder (Fig. 1). The powder samples were located on the bottom of the glass vessel for polymerization and conveniently stirred with a magnetic stirring system in order to allow a homogeneous coating of the powder. The radiofrequency (RF) generator (13.56 MHz) is connected to a copper coil which is enveloping the tubular glass vessel. The gases or monomers were supplied via a standard manifold with gas fluxes adjusted with needle valves. The system pressure was determined using a Pirani type vacuum meter (MKS, USA). The two stage mechanical pump (RV12 903, Edwards GB), positioned after the cold trap, evacuates the vessel to a reactor chamber pressure of 0.01 mbar.

All polymerizations were performed under continuous plasma. Different settings of the RF power were proved (10, 25, 50 and 100 W), and also different deposition times were tried (10, 30 and 60 min). Monomers for polymerization were used as supplied.

The monomer was preheated to reach the desired polymerization pressure. The monomer flow rate was set so the pressure within the reactor was 0,2 mbar.

Fig. 1. Picture of the down-stream tubular plasma reactor in the Materials Science Laboratory, IQS.

Acrylic acid (anhydrous, 99%, cat# 147230, Aldrich, Milwaukee, USA) was used as a monomer for the surface modification of the HA powder by plasma polymerization. In addition, 1,7-octadiene (98%, cat# 02501, Aldrich, Milwaukee, USA) was also employed as a crosslinker for its copolymerization with acrylic acid in order to produce films with higher solubility resistance to aqueous media.

HA powder was obtained by the sol–gel method described a previous work (Feranández, 2003) using triethylphosphite and calcium nitrate as precursors and performing an annealing treatment of 4 h at 400 °C.

2.1.2. Determination of the carboxylic groups content

A conventional back titration method was performed in order to determine the content of carboxylic functional groups present on the surface of the plasma modified HA samples. Briefly, samples were treated with an excess of 0.2 N sodium hydroxide (NaOH) solution for 2 h. The resultant solution was titrated with 0.4 N hydrochloric acid (HCl) solution. The carboxylic group content was expressed as moles of –COOH.

2.1.3. Determination of calcium release from the modified HA in simulated body fluid (SBF)

Modified and non-modified HA samples were compacted into disc-shaped pellets using a conventional press by pressing at 5–10 tonnes, obtaining discs of 13 mm diameter and approximately 1 mm height. Then, discs were soaked in SBF at 37 °C for a certain period. The concentration of calcium in the SBF was measured by atomic absorption spectroscopy at different time points in order to evaluate the interchange of calcium ions between the different samples and the SBF.

2.1.4. Preparation of the composite and evaluation of the composite viability to culture and differentiate mouse embryonic stem cells (mESC)

Non-modified and modified HA powders were used to prepare the composite together with the RAD16-I self-assembling peptide (BD^{TM} PuraMatrix^{TM} Peptide Hydrogel; peptide sequence: AcN-$(RADA)_4$-$CONH_2$); cat# 354250, BD Biosciences). RAD16-I peptide was at a concentration of 1% (10 mg/ml in 10% sucrose).

Solid HA particles were mixed with the liquid peptide in a proportion of 1:1 (wt.). The mixture was mechanically homogenized with a micropipette and sonicated for 10 min. The composite obtained was used to encapsulate cells at an approximately final concentration of 0.5% (w/v) with respect to the peptide content.

Download English Version:

https://daneshyari.com/en/article/14063

Download Persian Version:

https://daneshyari.com/article/14063

Daneshyari.com