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Human mesenchymal stem cell viability, proliferation and differentiation potential in response to ceramic chemistry and surface roughness

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Abstract

We investigated the effect of the ceramic chemistry and surface roughness of pure α-tricalcium phosphate, and also αTCP doped with either 1.5 wt% or 3.0 wt% of dicalcium silicate (C_2S), on the response of adult human mesenchymal stem cells (*ah*MSCs). A*h*MSCs were plated onto ceramic discs, prepared by a solid-state reaction. After being sintered, some samples were polished up to 1 μm, while others were kept as manufactured, which resulted in two surface roughness grades. Viability, proliferation and osteoinductive capacity were determined following various incubation periods.

The results showed a non-cytotoxic effect after an indirect apoptosis test. Cell adhesion and proliferation were surface roughness-sensitive and increased proportionally to the roughness of materials. These observations became more evident in the unpolished αTCP ceramic doped with 1.5 wt% C_2S, which induced osteoblastic differentiation as a result of the roughness and increased concentration of the C_2S solid solution in αTCP.

Keywords: A. Powder solid-state reaction; B. Surfaces; D. Apatite; E. Biomedical applications; Bone regeneration

1. Introduction

Surface reactivity is one of the common characteristics of bone bioactive ceramics. It contributes to their bone bonding ability and their enhancing effect on bone tissue formation. During implantation, reactions occur on the material tissue interface, which leads to time-dependent changes in the surface characteristics of the implant material, and also in tissues.

Modification of the biomaterial surface properties by controlling the characteristic length scale (e.g., grain size, crystal phase, etc.) or roughness is a promising approach to modulate selected cell functions.

Tricalcium phosphate, hydroxiapatite, bioglass and wollastonite are usually considered bone bioactive ceramics. These materials are generally bond to surrounding osseous tissue and enhance bone tissue formation [1–5]. Since direct bone bonding to Bioglass was first observed [6], considerable progress has been made in understanding the basic mechanisms of bone-biomaterial bond formation and its effect on bone formation. This progress has resulted mainly from two approaches: one focuses on studying the bone-biomaterials interface that was developed in vivo. An examination of the bonding zone revealed the consistent presence of an interfacial carbohydroxyapatite layer (CHA) [7,8]; in the second approach, *in vitro* immersions were

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used in simulated physiological fluids or media containing cells [9,10]. These analyses have revealed that some reactions, such as dissolution, precipitation and ion exchange, occur on implant material surfaces. The combination of *in vivo* and *in vitro* studies has led to a better understanding of the surface reactions of bioactive ceramics in the body and their effects on bone formation and cell function.

In general, bone substitute materials of different (natural or synthetic origins) can induce bone formation when combined with multipotent undifferentiated mesenchymal stem cells (hybrid materials). Based on this premise, several tissue engineering approaches have been used to increase the utility of biomaterials for clinical bone repair by including these osteoblastic precursor cells into a scaffold, followed by a period of osteogenic pre-differentiation of these cells in biomaterials before host implantation [11]. Presently, several techniques for the isolation and culture of *ah*MSCs obtained from bone marrow aspirates of human donors are available. Therefore, they represent an ideal source for their use in bone tissue bioengineering. To implement this approach, the selected biomaterial must be capable of allowing adhesion and growth of osteoprogenitor cells, and of promoting osteoblastic differentiation to thereby facilitate the neo-bone formation process.

Cells are able to discriminate subtle differences in surface roughness [12]. Osteoblast-like cells can discriminate between not only surfaces of different roughness, but also surfaces with comparable roughness, but with different topographies [13]. Thus surface morphology is of much importance.

The objectives of the present work were: (1) to evaluate the influence of substrate chemistry and (2) surface roughness on *ah*MSCs behaviour, and (3) to investigate how subtle differences in surface roughness can influence the *in vitro* short- and long-term responses of *ah*MSCs. For this purpose, we analysed the cell adhesion, proliferation, differentiation potential and viability of *ah*MSCs on bioceramic substrates of three chemistries (pure αTCP, and αTCP doped with either 1.5 wt% or 3.0 wt% of C_2S with a well-controlled grain size and crystal phase) with two different surface roughness values.

2. Material and methods

2.1. Material

The chemicals used in the synthesis of tricalcium phosphate and dicalcium silicate were calcium hydrogen phosphate anhydrous ($CaHPO_4 > 98.0$ wt%, Panreac, Barcelona, Spain), calcium carbonate ($CaCO_3 > 99.0$ wt% Fluka Analytical, St. Gallen, Switzerland) and high-purity silicon oxide ($SiO_2 > 99.7$ wt%, Strem Chemicals, Cambridge, England). Stoichiometric quantities of the raw powders to obtain tricalcium phosphate and dicalcium silicate were ground in a laboratory mixing miller (MM301-Retsch) using PSZ-zirconia balls.

2.2. Processing methods

TCP and C_2S were obtained by solid-state reaction sintering. Details of the technique can be found in previous publications [4,14].

The powders obtained were ground and characterised by X-ray fluorescence (XRF), X-ray diffraction (XRD-Bruker AXS D8-Advance X-ray Diffractometer; Karksruhe, Germany) and particle size distribution (Laser diffraction, Mastersizer S Malvern).

The αTCP ceramic and αTCP compositions doped with either 1.5 wt% or 3.0 wt% of C_2S (TCPss) were prepared for this study. The desired proportions of each component were weighed on an analytical balance, dispersed in acetone and thoroughly mixed in a manual agate mortar. The mixture was isostatically pressed into bars at 200 MPa, heated to 1500 °C for 2 h, and followed by liquid nitrogen quenching after rapid removal from the furnace. In order to homogenise compositions, bars were ground, pressed and reheated. This procedure was repeated 3 times. The powders obtained with an average particle size of 23 μm were pressed into bars at 200 MPa. The next step involved heating the bars up to 1500 °C for 4 h, followed by cooling to 1120 °C inside the furnace, where they remained for 16 h before being cooled down to room temperature. Heat treatment temperatures were carefully selected after bearing in mind the information provided by the αTCP_{ss}-silicocarnotite subsystem within the binary system of TCP–C_2S [$Ca_3(PO_4)_2$–Ca_2SiO_4] [15]. The final samples were cut from the obtained bars, which measured 7 mm in diameter and 1.5 mm in thickness.

Two different surface roughness types were produced: the first performed by a cutting machine, with which an opaque surface was obtained; the second produced by sequential polishing with 6 μm and 1 μm diamond pastes, with which a bright mirror-like surface was obtained.

2.3. Material and surface characterisation

2.3.1. Powder X-ray diffraction

XRD were obtained using an automated diffractometer. They were compared with the database provided by the Joint Committee on Powder Diffraction Standards (JCPDS).

2.3.2. Surface profilometry

The surface roughness of the pure αTCP, and also the αTCP doped with either 1.5 wt% or 3.0 wt% of C_2S disks, was measured by profilometry in a Mitutoyo SJ-201P profilometer. Five disks of each material with the different roughness values were measured to obtain an average roughness value R_a. Five individual measurements were taken on each specimen. The statistically significant differences in the Ra values were determined by an ANOVA statistical analysis. Differences were considered significant when $p < 0.05$.

2.3.3. Scanning electron microscopy

Scanning electron microscopy (SEM-Hitachi S-3500N), equipped with an Energy Dispersive X-ray Spectrometer (EDX) microanalysis probe (INCA-Oxford), was used to investigate the morphology and texture of surfaces, and the semiquantitative analysis of surface composition.