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In vitro behaviour of adult mesenchymal stem cells seeded on a bioactive glass ceramic in the SiO_2–CaO–P_2O_5 system

L. Meseguer-Olmo [a,*], A. Bernabeu-Esclapez [a], E. Ros-Martinez [a], S. Sánchez-Salcedo [b], S. Padilla [b], A.I. Martín [b], M. Vallet-Regí [b], M. Clavel-Sainz [a], F. Lopez-Prats [a], C.L. Meseguer-Ortiz [a]

[a] *Unidad de Investigación Experimental, Laboratorio de Cirugía Ortopédica Experimental, Hospital Universitario V, Arrixaca, Universidad de Murcia, 30520 Murcia, Spain*
[b] *Departamento de Química Inorgánica y Bioinorgánica, F. Farmacia, Universidad Complutense de Madrid, 28040 Madrid, Spain*

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Abstract

This work describes the evaluation of a glass ceramic (55S41C4P-1300) as a potential substrate for bone tissue engineering. For that purpose, the capacity of mesenchymal stem cells (MSCs), isolated from rabbit bone marrow, to adhere, proliferate and differentiate into osteoblast (OBs) with or without 55S41C4P-1300 was investigated. Two types of culture medium, i.e. growth medium (GM) and osteogenic medium (OM), were evaluated. The bioactive 55S41C4P-1300, containing pseudowollastonite, wollastonite, tricalcium phosphate and crystoballite as crystalline phases, was obtained by heat treatment of a sol–gel glass ($55SiO_2$, 41CaO, $4P_2O_5$ (mol.%)) at 1300 °C.

The results showed that the MSCs adhered, spread, proliferated and produced mineralized extracellular matrix on 55S41C4P-1300 regardless of the culture medium used. As the same time, they showed an osteoblastic phenotype, and this phenomenon was accompanied by the gradual diminution of the marker CD90 expression. The 55S41C4P-1300 was able to induce the differentiation of MSCs into OBs in the same way as OM without glass ceramic. This effect increased with the combination of 55S41C4P-1300 with OM. The glass ceramic evaluated in this work is bioactive, cytocompatible and capable of promoting the differentiation of MSCs into OBs. For that reason, it could be regarded as a suitable matrix in tissue engineering for bone tissue regeneration.

Keywords: Glass ceramic; Scaffold; Bone tissue engineering; Mesenchymal stem cell; Osteoblast

1. Introduction

The treatment of large bone defects of any aetiology (infection, traumatism, tumours, malformations, pseudoarthrosis, etc.) is still a challenge in the wide field of reconstructive osseous surgery involving orthopaedic, oral and maxillary–face–skull surgeons, and hence is the subject of great discussion. The advances in all areas of medicine in last decades and the better understanding of the biology of bone tissue repair have led to the consideration of new strategies in order to achieve tissue regeneration.

For this reason, in recent years much interest has focused on improving the osteogenic properties of biomaterials by means of the construction of hybrid materials that are biologically active. To this end, different types of cells [1–4] have been seeded onto different materials to act as scaffolds which can optimize the repair results [1,2,5–9] by mimicking the structure and the biology of autologous spongy bone. The scaffolds used for this purpose must fulfil a number of basic requirements, such as biocompatibility, mechanical integrity and osteoconductivity. In addition, they must influence the genes in the bone-

* Corresponding author. Tel.: +34 968 241826.
E-mail address: meseguer@arrakis.es (L. Meseguer-Olmo).

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generating cells to enable efficient cell differentiation and proliferation [10,11].

It is known that, since the postnatal period, bone marrow presents precursor cell populations involved in the maintenance of connective tissue, such as adult mesenchymal stem cells (MSCs). These cells are characterized by their capacity to autorenew and differentiate into multiple mesenchymal cell lines, e.g. adipocytes, osteoblasts, chondrocytes, myocytes and fibroblasts [12–15]. This type of cell has also been isolated from the periosteum, veins, adipose tissue, etc. of several species other than human, such as rat, rabbit and mouse. Their osteogenic potential and their capacity to differentiate into osteogenic cells have been proven [16–19]. This highlights the potential of these bone marrow cells as suitable elements to improve the osteogenic and osteoinductive capacity of these materials.

Of the biomaterials used as scaffolds for bone regeneration, bioactive glasses are very interesting because they are bioactive, resorbable and also osteoproductive [20]. They exert genetic control over the osteoblastic cell cycle and rapidly express genes to regulate osteogenesis and the production of growth factor [10,11]. In previous work, we have demonstrated that by calcination of sol–gel glasses it is possible to obtain glass ceramic (GC) with better mechanical properties than glasses without detriment of their fast bioactive behaviour [21,22]. In order to use this materials as scaffolds for tissue engineering we have obtained scaffolds with a network of designed three-dimensional interconnected macropores of 400–500 μm in diameter [23]. In this work a GC with a composition (in mol.%) of $55SiO_2$, 41CaO, $4P_2O_5$, was investigated. This GC shows a fast in vitro bioactive behaviour, i.e. an apatite layer covered the material surface after 3 days soaking in simulated body fluids (SBF) [21–23]. With the goal in mind of using this material as a scaffold for tissue engineering, the aims of our work were:

(1) To investigate if the adult MSCs were able to adhere, proliferate and differentiate or express phenotypical differentiation of osteoblasts (OBs) on this bioactive GC.
(2) To evaluate in vitro the effect of two culture media (growth and osteogenic) on the phenotypical differentiation into OBs of primary MSCs line. For this purpose the behaviour of the MSCs was compared with a control culture consisting of OBs.

In this work, we hypothesize that a biomaterial combining MSCs and a bioactive GC can reach the precise requirements to be considered as an osteoinductive and osteogenic material.

2. Materials and methods

2.1. Material preparation and characterization

The GC was obtained by heat treatment at 1300 °C of a bioactive glass (nominal composition (mol.%): $55SiO_2$, 41CaO, $4P_2O_5$) obtained by the sol–gel method [24]. Stoichiometric amounts of tetraethyl orthosilicate, calcium nitrate tetrahydrate and triethyl phosphate (all of them from Aldrich) were used as precursors of the corresponding oxides. The obtained dried gel was milled and sieved to obtain the fraction of particles with sizes ranging from 32 to 63 μm. The pieces were obtained by compacting 0.1 g of dried gel by uniaxial pressing (55 MPa) followed by isostatic pressing (150 MPa). The obtained pellets were heated to 1100 °C (5 °C min^{-1}) for 3 h. Subsequently the pieces were sintered at 1300 °C (5 °C min^{-1}) for 3 h. Finally, pieces of 4.8 mm diameter and 1.3 mm height were obtained. Throughout the manuscript, the obtained material will be referred to as 55S45C4P-1300.

The characterization of the material 55S41C4P-1300 showed that the crystalline phases present in this GC were pseudowollastonite (54 wt.%, JCPD No. 74-0874), wollastonite (38 wt.%, JCPD No. 76-0186), tricalcium phosphate (4 wt.%, JCPD No. 70-0364) and crystobalite (4 wt.%, JCPD No. 76-0936).

Before cell culture studies the pieces were cleaned applying pressured air and rinsed several times with sterile PBS. After that, they were dried at 37 °C and finally the pieces were sterilized by plasma gas.

2.2. Preparation and culture of adult MSCs

2.2.1. Primary culture: isolation and culture of MSCs

The adult MSCs were isolated from bone marrow of adult rabbits obtained by direct aspirations of ileac crest. For the isolation, the aspirated material was settled in a tube containing sodium heparin (20 U ml^{-1} of aspirated material), then it was passed through a nylon mesh of 100 μm to obtain individual cells. The cell suspension was incubated with 0.16 M ammonium chloride to lyse erythrocytes and then centrifuged at 200*g* for 10 min. After estimating the viability with tripan blue, the cells were plated out in 75 cm^2 culture flacks (Sarsted) with 10 ml of standard culture medium and they were incubated at 37 °C, in a 5% CO_2 atmosphere and 95% of relative humidity. The culture medium used was α-minimal essential medium (MEM; Gibco) supplemented with 15% of fetal calf serum (FCS, Gibco) and routine antibiotics (100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin; Gibco).

For the experiments with rabbits the institutional ethical protocols for the protection of experimentation animals (R.D. 223/1988 de 14 Marzo y orden 13 octubre de 1989, Spain) were complied.

2.2.2. Subcultures of MSCs

After 7 days, the culture medium was renewed removing thus the non-adherent hematopoietic cells and selecting the MSCs given their proved capacity of attaching to the plastic of culture flasks [25]. Once the cells were confluent (7 days later), they were subcultured in a 1:3 proportion treating the culture flask with trypsin/EDTA (0.25%/0.25%) in phosphate-buffered saline (PBS, pH 7.4) for 5 min.

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