Influence of topography of porous – Nurse´s A phase bioceramic on metabolic activity of adult human mesenchymal stem cells.

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Introduction

It is well known that Si-Ca-P bioceramics are promising bioactive materials for bone tissue engineering, particularly for bone repair. Bone tissue mineral is a calcium-phosphate (CaP)-based apatite phase. For this reason, CaP ceramics—typically hydroxyapatite (HA) and tricalcium phosphate (TCP)—are widely used for bone tissue replacement[1]. Silicon incorporation to these ceramics may be fundamental to skeletal development stimulating mesenchymal stem cells proliferation and differentiation to osteoblasts[2].

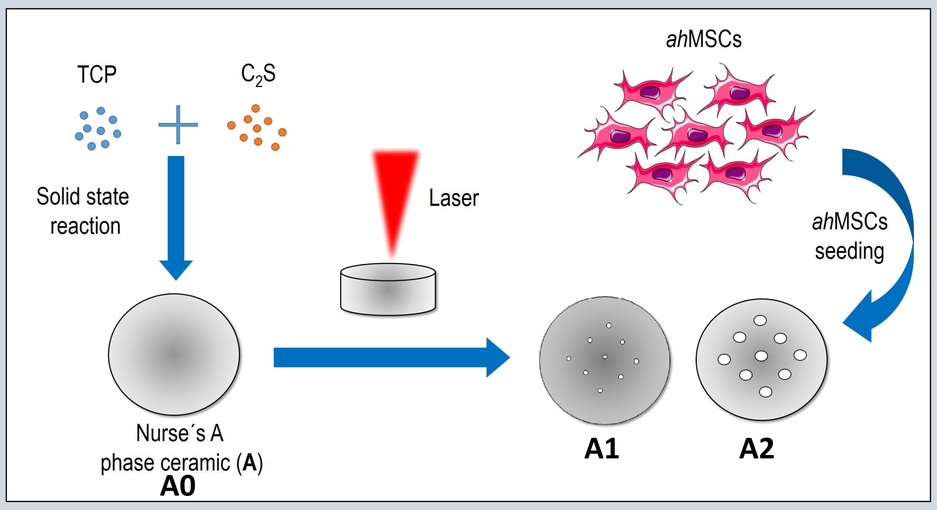
An important characteristic of a bioceramic designed for bone reconstruction is its open porosity with a high area / volume ratio that facilitates the initial circulation of biological fluids, cell migration and vascular invasion after bone implantation[3].

In this work, we have used a laser surface treatment to create porosity in a Si-Ca-P single phase ceramic and we have tested if the increase of the surface area and the pore size affect to adult mesenchymal stem cells viability and proliferation.

Material and methods

A Si–Ca–P (Nurse's A phase, A) single-phase ceramic material was prepared from tricalcium phosphate and dicalcium silicate ceramics, which were used as starting materials.

Discs-shaped Nurse's A-phase samples (**A0**), measuring 7 mm in diameter and 3 mm in length, were cut from the sintered bulk with a diamond saw.



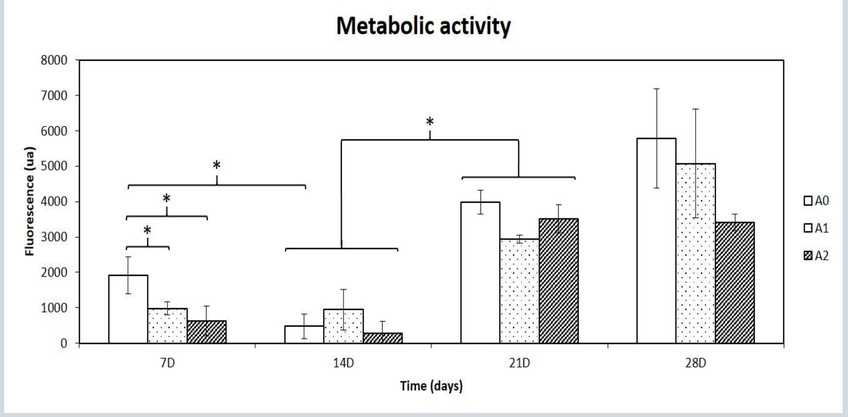
Nurse's A phase (**A0**) surfaces were processed using a Q-switched Nd:YAG laser.

- Parameters: frequency (2 KHz), electrical current (35 A), speed (5mm/s).
- Pore diameter: 9 (3x3) pores x 70 µm (**A1**) / 9 (3x3) pores x 350 µm (**A2**)

To assess the biological properties and the biocompatibility of the porous Nurse's A phase ceramic, adult human mesenchymal stem cells (*ah*MSCs) were seeded onto the top of A0, A1 and A2. After that, Scanning Electron Microscopy (SEM) images were taken after 28 days of the seeding and a metabolic activity assay was run (Alamar Blue™ assay, Invitrogen) at 7, 14, 21 y 28 days after the seeding to test the cellular proliferation.

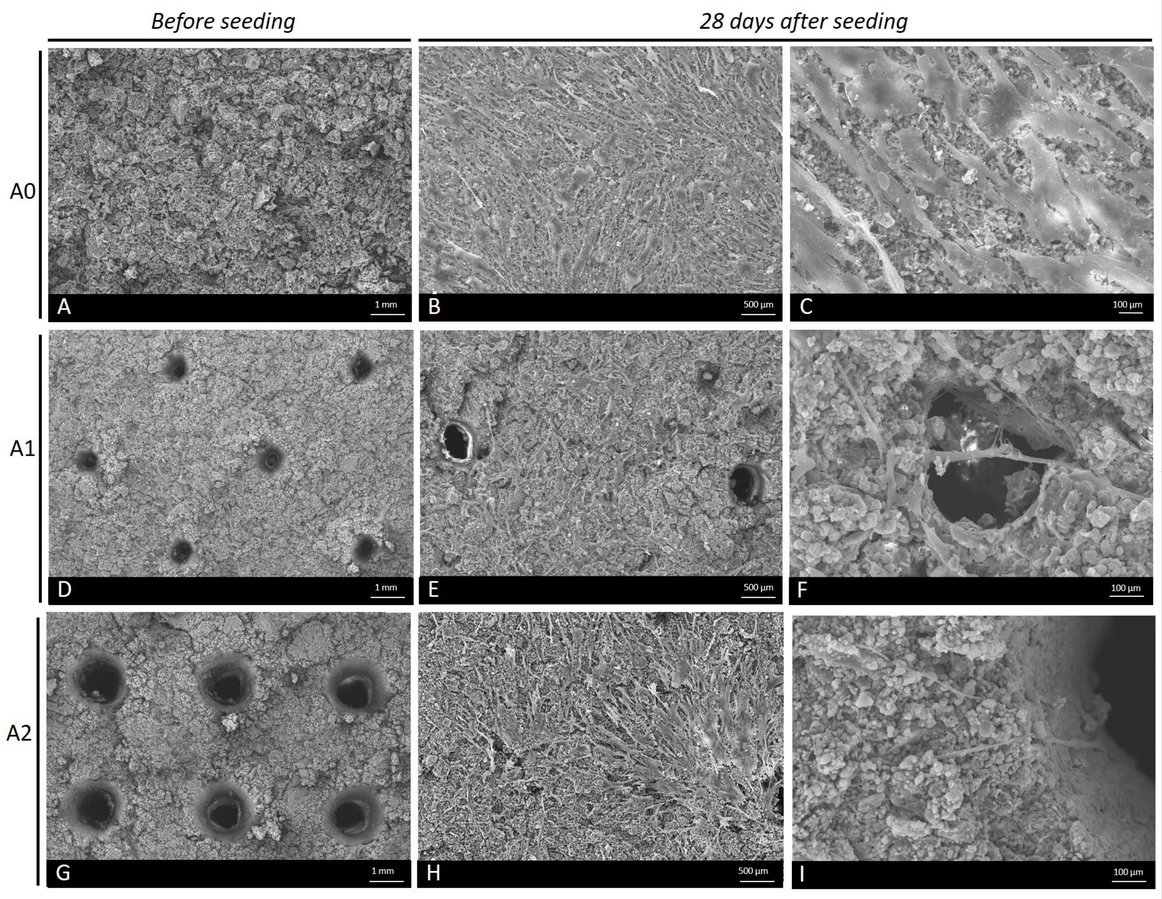
Results

Alamar Blue Assay



- Alamar Blue assay showed that A0, A1 and A2 were biocompatible with *ah*MSCs and stimulate cellular proliferation.
- Metabolic activity differences were not found between *ah*MSCs seeded on A0, A1 and A2.
- After 1 and 2 weeks, metabolic activity of *ah*MSCs were very low. It was necessary 3 weeks for *ah*MSCs proliferation.

Scanning Electron Microscope (SEM)



- A Si–Ca–P (Nurse's A phase, **A0**) single-phase ceramic material was obtained. A0 was processed and 70 µm and 350 µm porous Nurse´s A phase were developed (**A1** & **A2** respectively) (A,D and G images).
- SEM images of the bioceramics verify that surface scaffolds were well covered by *ah*MSCs after 28 days of the seeding (B, E and H).
- *ah*MSCs showed a fibroblastic appearance, stretched and covering much of the surface. There were not cytotoxicity signals or morphological alterations throughout the study (C).
- *ah*MSCs seeded on A1 could cross the pores with philopodia. In A2 some cells were observed entering into the pores (F and I).

Conclusions

In this work, we have developed a biocompatible Si-Ca-P ceramic with 70 µm and 350 µm pores. The increased surface área and the pore size didn´t affect to *ah*MSCs proliferation.

In vitro assays with *ah*MSCs showed that cells could proliferate in the surface of the scaffolds, and they could cross and enter into the pores. So, if we use these bioceramics *in vivo*, we could probably observe how the cells penetrate into the bioceramics through the pores, improving the cellular and vascular colonization of the biomaterial and allowing initial circulation of the body fluids from the first moment of implantation, increasing the bioceramic biodegradability.

Due to the success of these trials, the next experiments will be orientated to create bioceramics combining different sized pores interconnected in order to mimic the natural structure of bone.

References

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Acknowledgements

Part of this work has been supported by Catholic University of Saint Anthony (UCAM) contract grant number: PMAFI/04/15

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