Surface topography modification of multilayer 3D ceramic scaffolds fabricated by the sol-gel method

* Sebastián Eduardo [1], Murciano Ángel [2], De Aza Piedad [1], Velásquez Pablo [1]

[1] Instituto de Bioingeniería, Universidad Miguel Hernández, Avda. ferrocarril s/n, Elche, Alicante, 03202, Spain
[2] Departamento de Materiales, Óptica y Tecnología Electrónica, Universidad Miguel Hernández, Avda. Universidad s/n, Elche, Alicante, 03202, Spain

* E-mail: eduardo.sebastian@goumh.umh.es

INTRODUCTION

Three-dimensional (3D) calcium phosphate (CaP) porous scaffolds offer a promising alternative for the treatment of damaged bone tissue (1). The properties of the scaffold, including the pore size and structure, chemical composition, surface topography, degradability rate and mechanical strength are essential for the successful osseointegration of the scaffold.

Surface topographic patterns like ridges and grooves can promote osteoblast adhesion, alignment, proliferation and differentiation by mimicking the biophysical cues provided by the collagen fibers that are part of the bone extracellular matrix (ECM) *in vivo* (2).

OBJECTIVES

- Fabrication and characterization of multilayer 3D CaP porous scaffolds.
- Surface topography modification by introduction of a topographic pattern (ridges and grooves).
- Characterization of the modified scaffolds and *in vitro* bioactivity tests.

MATERIALS AND METHODS

Multilayer CaP scaffolds were obtained by sol-gel in combination with the polymeric sponge replication method (3). Different sol-gel compositions were prepared to obtain layers with different mechanical and bioactive properties. Topographic modifications on the scaffolds surface were conducted by a chemical etching treatment with different times (30 s, 60 s, 120 s) and concentrations (3 %, 5 %, 10 %) of acetic acid (CH_3COOH).

The obtained scaffolds were characterized by Scanning Electron Microscopy with Energy Dispersive X-Ray Spectroscopy (SEM-EDX), X-Ray Diffraction (XRD), Mercury Porosimetry and Digital Holographic Microscopy (DHM). Mechanical strength was tested with a Manual Test Stand and the *in vitro* bioactive capacity of scaffolds was determined by immersion in simulated body fluid (SBF) (4) for 7, 14 and 21 days.

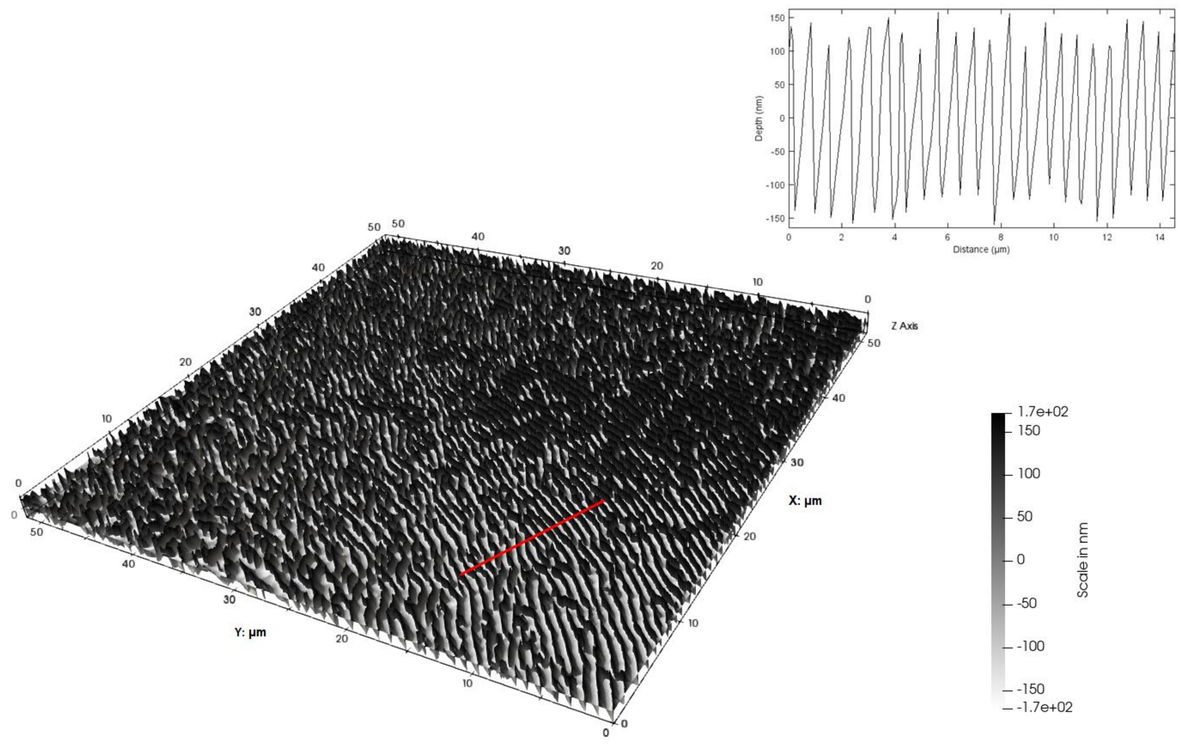

Fig. 4. 3D surface profile of etched scaffolds analyzed by DHM after 120s of etching time.

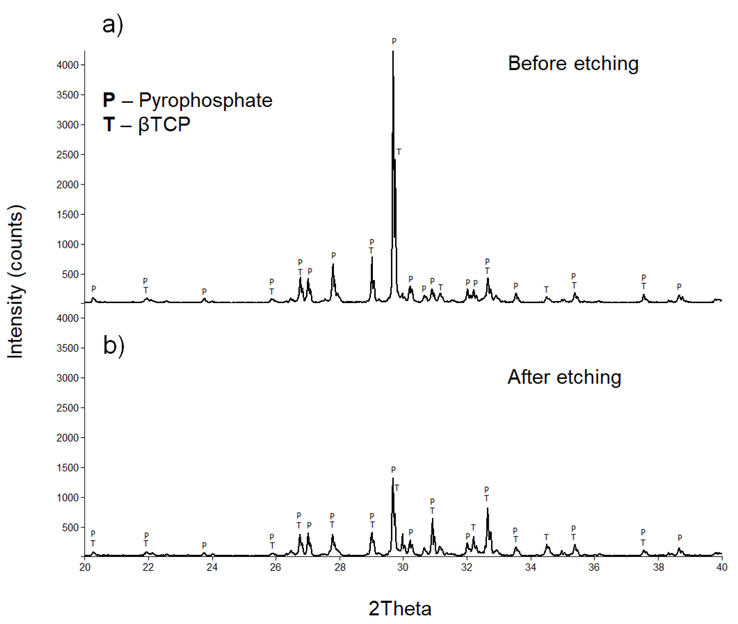

Fig. 3. XRD patterns of the scaffolds' phases, pyrophosphate and β-tricalcium phosphate, before (a) and after (b) etching.

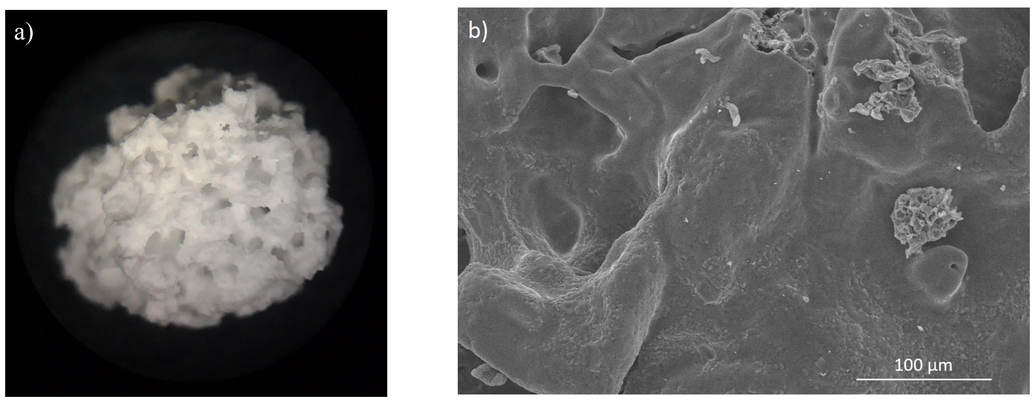

Fig. 1. Macrostructure of the multilayer CaP scaffolds (a) and SEM micrograph of their microstructure (b).

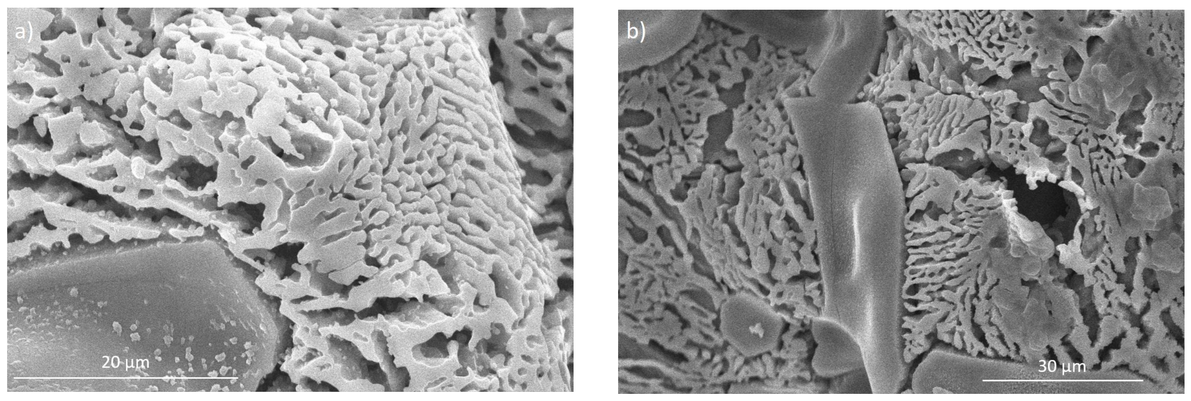

Fig. 2. SEM micrographs of chemically etched scaffolds at 3% acetic acid concentration: a) after 60s; b) after 120s.

RESULTS

The microstructure and mineralogical composition of the multilayer 3D CaP porous scaffolds was characterized by SEM-EDX (Fig. 1B) and XRD (Fig. 3A) respectively. Scaffolds presented an interconnected porosity (Fig. 1A), and their surface was smooth with some hexagonal grains of pyrophosphate ($Ca_2O_7P_2$) and β-tricalcium phosphate (β-TCP) (β-$Ca_3(PO_4)_2$). The XRD patterns of scaffolds confirmed that the main constituent phases were pyrophosphate (COD-96-151-7239) and β-tricalcium phosphate (COD-96-151-7239).

Scaffolds were chemically etched to modify their surface and create a topographic pattern in the form of ridges and grooves. A 3% acetic acid concentration for 120s was selected as appropriate to obtain a homogeneous distribution of ridges and grooves along the scaffolds' surface. The resulting scaffolds were characterized by SEM-EDX (Fig. 2), XRD (Fig. 3B) and DHM (Fig. 4).

The EDX results revealed that the scaffolds Ca/P ratio was of about 1.5 (Ca/P ratio for β-TCP) on the grooved areas. The XRD patterns after etching (Fig. 3B) showed that the peaks corresponding to pyrophosphate (COD-96-151-7239) were diminished while the peaks for β-tricalcium phosphate (COD-96-151-7239) were more prominent, probably due to the dissolution of the pyrophosphate phase as a consequence of the chemical attack.

The main dimensions, width and depth, of the ridges and grooves were determined by DHM (Fig. 4). Their dimensions were around 900 nm – 2 µm in width and 200 nm – 300 nm in depth and varied with etching time. Scaffolds' compressive strength was measured before and after chemical etching. The untreated scaffolds mechanical strength was of 0,46 – 0,67 MPa, while their resistance was of around 0,56 – 0,67 MPa after treatment. Chemical etching did not alter the scaffolds' mechanical properties.

Scaffolds' *in vitro* bioactivity was tested by soaking them in SBF for 7, 14 and 21 days in order to determine if the etching process affected their bioactive capacity. After each time period, scaffolds were analyzed by SEM (Fig. 5). After 14 days and 21 days, spherical precipitates were deposited on scaffolds' surface and their Ca/P ratio was of 1.67, corresponding to hydroxyapatite ($Ca_5(PO_4)_3(OH)$), confirming that chemical etching did not affect the scaffolds' bioactive capacity.

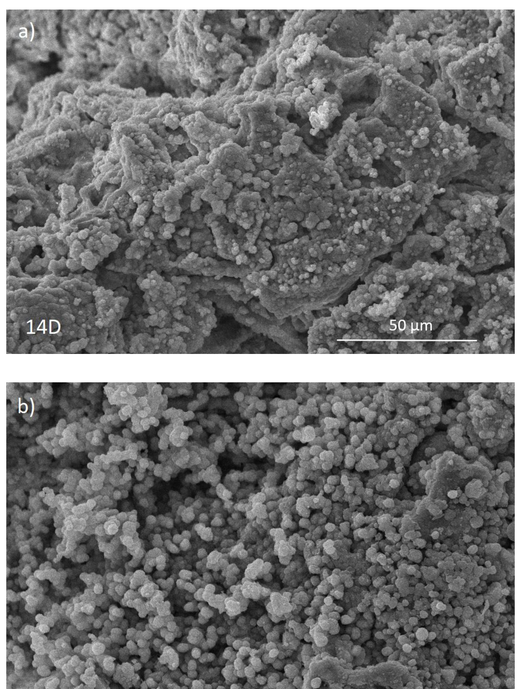

Fig. 5. SEM micrographs of the scaffolds after different immersion times in SBF: a) 14 days; b) 21 days.

CONCLUSIONS

Multilayer 3D CaP porous scaffolds were developed to be used in bone tissue engineering. After their physical and chemical characterization, a chemical etching treatment was applied to scaffolds in order to modify their surface topography and introduce a topographic pattern in the form of rides and grooves. Surface patterns like ridges and grooves can promote osteoblast adhesion, alignment, proliferation and differentiation (2), therefore, enhancing the osseointegration of the scaffolds *in vivo*.

REFERENCES

1. Denry I., Kuhn L. (2016) Design and characterization of calcium phosphate ceramic scaffolds for bone tissue engineering. *Dental Materials*, 32, 43–53.
2. Wang P., Zhao L., Liu J., Weir M., Zhou X., Xu H. (2014) Bone tissue engineering via nanostructured calcium phosphate biomaterials and stem cells. *Bone Res*, 2, 14017.
3. Ros-Tárraga P., Murciano A., Mazón P., Gehrke S., De Aza P.N. (2017) New 3D stratified Si-Ca-P porous scaffolds obtained by sol-gel and polymer replica method: Microstructural, mineralogical and chemical characterization. *Ceramics International*, 43, 6548–6553.
4. Kokubo T., Takadama H. (2006) How useful is SBF in predicting in vivo bone bioactivity?. *Biomaterials*, 27(15), 2907-2915.

Acknowledgments

This work is part of the project PID2020-116693RB-C21 funded by MCIN/AEI/ 10.13039/501100011033