Versatility of the Sol-Gel method in obtaining magnesium-doped multilayer ceramic scaffolds with different properties for applications in bone tissue engineering

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INTRODUCTION

Calcium phosphate ceramic scaffolds are ideal for substitution of diseased or fracture bone. However, in order to obtain scaffolds that meet all the necessary requirements for their application in tissue engineering, the development of multilayer scaffold is proposed. The structure are composed of a porous and resistant core, which is coated with layers of a second chemical composition that provides bioactivity. In this research, in addition to developing multilayer scaffolds, a comparative study is carried out between scaffolds consisting of cores with different crystalline phases and magnesium-doped coatings. The incorporation of magnesium ions in the coatings is because this ion improve cell adhesion and stimulate the formation of blood vessels[1-2].

MATERIALS & METHODS

The scaffold cores were obtained using a Sol-Gel solution with the following chemical composition **SiO_2 - 25P_2O_5 – 68CaO – 6Li_2O** (% molar). Once the reagents were mixed, the solution was heated at 100 °C for 15 minutes and 1 hour. Subsequently, the polyurethane sponges were immersed in each solution and after several coatings (10 and 30 times), the samples were sintered at 950 °C. Finally, new solutions were prepared with the following chemical composition **29SiO_2 – 3P_2O_5 – (68-x)CaO – xMgO** (% molar) [x = 0.7, 2, 7], corresponding to the external coating layers. The previously prepared cores were coated 6 times with each solution and again sintered at 950 °C. The scaffolds were mineralogically, chemically and physically characterized using different techniques such as X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM-EDX) and simple compression test. In vitro bioactivity was evaluated by immersion in simulated body fluid (SBF) prepared according to ISO/FDIS 23317:2017.

RESULTS & DISCUSSION

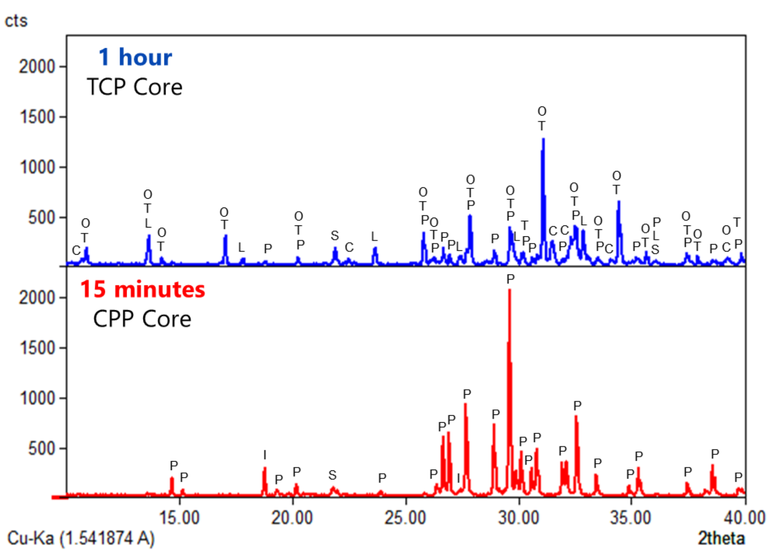

Figure 1.- XRD diffraction patterns of the cores with solutions heated for 1 hour and 15 minutes. Two cores were obtained using the same chemical composition and varying the heating time of Sol-Gel solution. The solution heated for 1 hour (blue) resulted in a core whose main crystalline phase is tricalcium phosphate (TCP, **T: β-$Ca_3(PO_4)_2$**). While the solution heated for 15 minutes (red) resulted in a core consisting mainly by calcium pyrophosphate (CPP, **P: $Ca_2P_2O_7$**). In addition, to these main phases, the following phases were identified: **I: $Li(PO_3)$, S: SiO_2, L: $LiCa(PO_4)$, O: $Ca_{9.95}Li_{1.05}(PO_4)_7$, C: $Ca_5(PO_4)_3Cl$**.

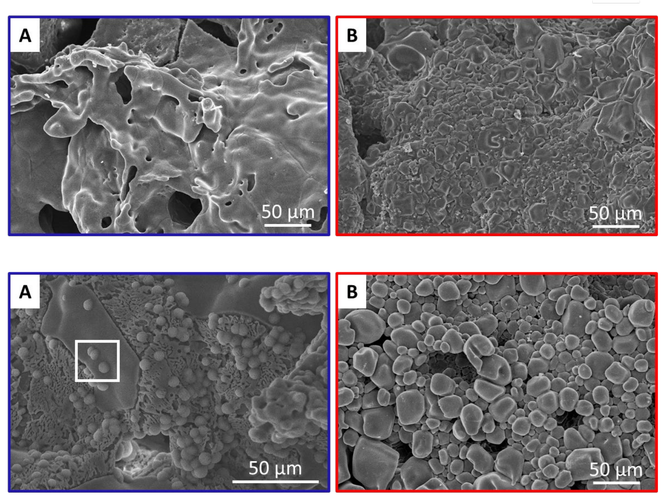

Figure 2.- SEM images of the TCP (A) and CPP (B) cores. The CPP core showed hexagonal grains with a Ca/P ration of 0.93-1.06. In addition to a vitreous phase formed between the grains with a Ca/P ration of 0.25-0.35. While the TCP core showed a smooth surface without grains and Ca/P ratio of 1.06-1.40.

Figure 3.- SEM images of TCP (A) and CPP (B) core after 14 days in SBF. TCP core showed apatite precipitates after 14 days and lamellar structure formation. While CPP core did not show in vitro bioactivity during the immersion in SBF. Instead showed degradation of the vitreous phase.

Both cores showed comparable compressive strength to trabecular bone (1.5-9.3 MPa)[3]: **(1.03 ± 0.50)MPa** for TCP core and **(2.87 ± 1.19)MPa** for CPP core.

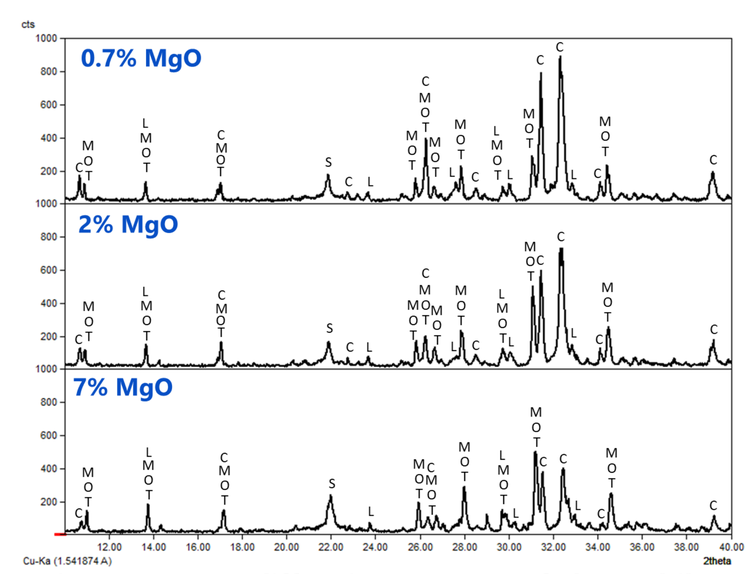

Figure 4.- XRD diffraction patterns of the multilayer scaffolds with TCP core coated and doped with 0.7, 2 and 7% of MgO. (M: $Ca_{10.115}Mg_{0.385}(PO_4)_7$).

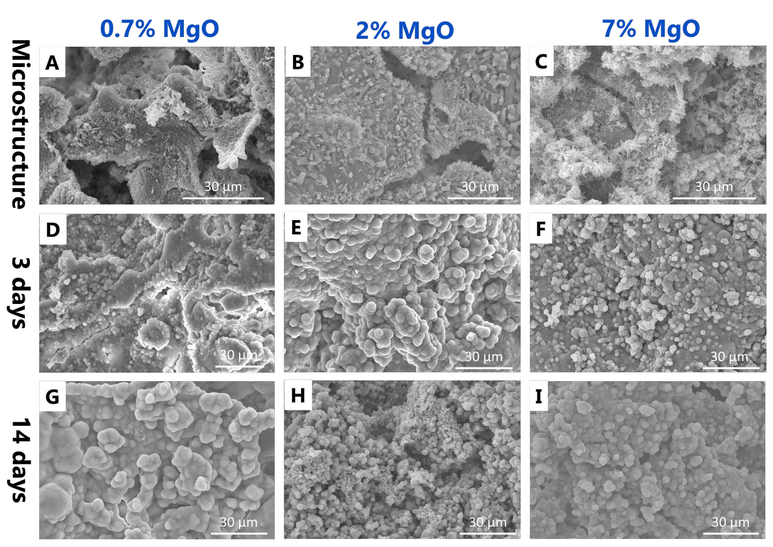

Figure 5.-SEM images of microstructure (A-C) and after 3 (D-F) and 14 days (G-I) in SBF of the multilayer scaffolds with TCP core coated and doped with 0.7, 2 and 7% of MgO.

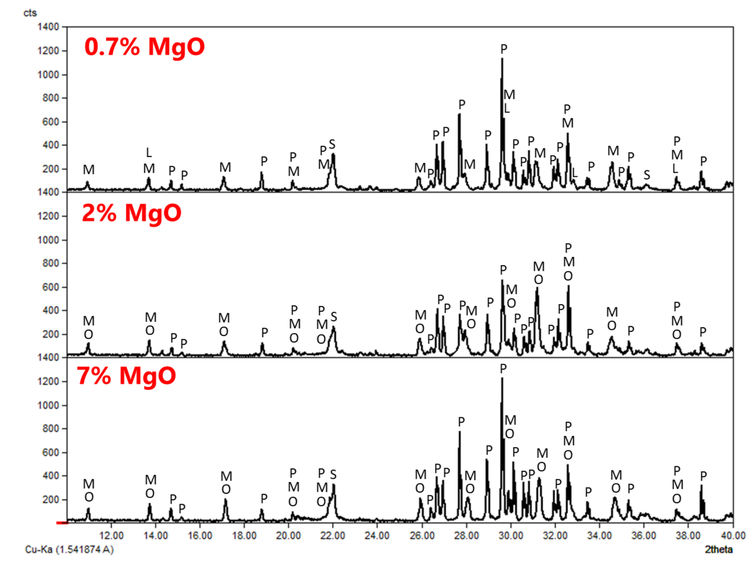

Figure 6.- XRD diffraction patterns of the multilayer scaffolds consisting of CPP core coated and doped with 0.7, 2 and 7% of MgO. (M: $Ca_{10.115}Mg_{0.385}(PO_4)_7$).

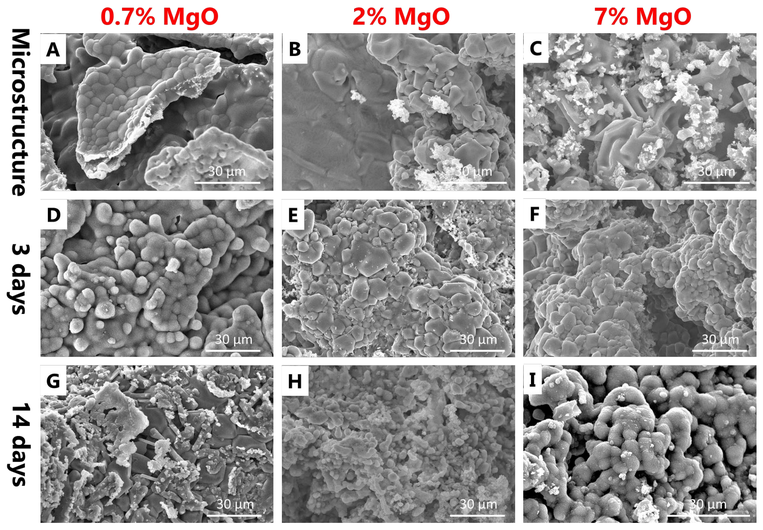

Figure 7.-SEM images of microstructure (A-C) and after 3 (D-F) and 14 days (G-I) in SBF of the multilayer scaffolds with CPP core coated and doped with 0.7, 2 and 7% of MgO.

Core TCP + Mg-doped coatings

- The phase $Ca_{10.115}Mg_{0.385}(PO_4)_7$ was identified (**Figure 4**). It was observed that CPP phase and $Ca_5(PO_4)_3Cl$ phase decreased with increasing MgO concentration. In the presence of Mg, the TCP phase stabilize instead of CPP phase.
- On the surface of the scaffolds coating plates and particles of different sizes are observed (**Figure 5(A-C)**). EDX analysis identified the presence of calcium, phosphorus, chlorine, magnesium and silicon.
- Multilayer scaffolds with TCP core showed in vitro bioactivity with apatite precipitates from day 3 of immersion in SBF (Figure 5(D-F)), independently of the dopant concentration.

Core CPP + Mg-doped coatings

- The formation of the following phases was identified: $Ca_{10.115}Mg_{0.385}(PO_4)_7$, $LiCa(PO_4)$ and $Ca_{9.95}Li_{1.05}(PO_4)_7$ (**Figure 6**). In the presence of Mg, the vitreous phase dissolved and reacts with lithium. While CPP transformed to TCP.
- It was observed that as MgO concentration increased, irregular particles formed on the scaffold surface (**Figure 7(A-C)**).
- The scaffold doped with 0.7% MgO presented apatite precipitate at 3 days (**Figure 7D**) that disappeared later (**Figure 7G**). While the scaffolds doped with 2% MgO and 7% MgO presented apatite precipitate after 14 days (**Figure 7H and 7I**). The instability of the apatite precipitate is due to the chelating effect of the phosphate chains in the vitreous phase, which remove the calcium and thus the precipitate. It was observed that as MgO concentration increased, amorphous apatite precipitates are formed.

CONCLUSIONS

Two types of cores for the multilayer scaffolds were obtained thanks to the versatility of the sol-gel process, both with mechanical strength comparable to trabecular bone. Despite not showing adequate in vitro bioactivity, the coatings were able to considerably increase bioactivity. Moreover, the scaffolds contain Mg, which is known to improve cell adhesion. The core not only serves as a mechanical support for the coatings but also its crystalline phases influence bioactivity. Scaffolds with TCP core show stable bioactivity from day 3. While scaffold with CPP core show time-varying bioactivity due to the vitreous phase. These characteristics make the scaffolds very promising for tissue engineering applications.

ACKNOWLEDGEMENT

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