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Vat photopolymerization of biomimetic bone scaffolds based on Mg, Sr, Zn-substituted hydroxyapatite: Effect of sintering temperature

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Introduction: The incidence of bone fractures is globally increasing, with substantial economic implications. Bone grafting, performed at a global scale with over 2 million cases annually, ranks as the second most common tissue transplantation procedure after blood transfusions. Autografts, allografts, and bone graft substitutes are the primary categories used for treating critical-sized bone defects (> 2 cm), but they come with limitations and risks such as donor site morbidity, postoperative pain, and infection. In response, the development of synthetic bone scaffolds has been actively pursued, aiming to provide temporary mechanical support for tissue regeneration while promoting osteogenesis. Despite challenges in replicating bone's intricate characteristics, recent advancements in printing technologies offer breakthroughs in biomimetic scaffold development for bone regeneration, allowing precise recreation of bone-like structures and integration of biological factors. Ceramic vat photopolymerization enables fabrication of intricate porous structures closely mimicking native bone architecture. Synthetic hydroxyapatite (HAp), due to its similarity to bone constituents, particularly stands out as an ideal material for scaffolds. Incorporating trace elements into HAp can enhance its properties (e.g. promoting bone regeneration). Present study explores novel HAp-based scaffolds substituted with trace elements and fabricated using ceramic vat photopolymerization. The effects of the selected trace elements (Sr, Mg and Zn) and the sintering temperatures (900, 1000, 1100, 1200, and 1300 °C) were investigated regarding the crystalline phase content, elemental distribution, microstructure, thermal stability, and mechanical properties

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Phase composition and microstructure of fabricated scaffolds

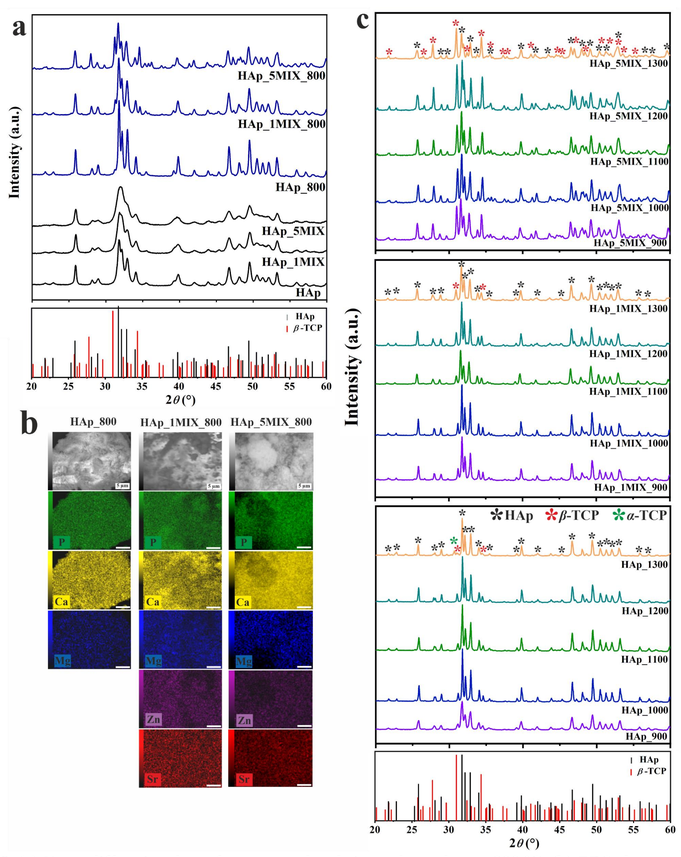

Figure 2. (a) X-ray diffraction data of as-prepared and sintered powders at 800 ºC. (b) EDS element mapping of the HAp_800, HAp_1MIX_800, and HAp_5MIX_800 powder samples. Scale bar: 5 µm. (c) X-ray diffraction data of fabricated scaffolds sintered at different temperatures.

- The XRD peaks of the as-prepared powders closely match the line patterns for HAp. After heat treatment at 800 ºC, HAp_800 powders exhibited a strong resemblance to the HAp pattern, while in the case of HAp_1MIX_800 and HAp_5MIX_800 powders, additional peaks corresponding to β-tricalcium phosphate (β-TCP) were observed (Fig. 2a). The EDS elemental mapping (Fig. 2b) confirmed the presence and even distribution of calcium, phosphorus, magnesium, strontium and zinc.
- Scaffolds show similar XRD patterns to the powders with a notable increase in the intensity of the characteristic β-TCP peaks, especially at higher sintering temperatures and substitution levels.

Materials and Methods

- The multi-substituted (Sr^{2+}, Mg^{2+} and Zn^{2+}) and non-substituted reference HAp powders were synthesized by the wet precipitation method.
- The selected (Sr + Zn + Mg)/(Ca + Sr + Zn + Mg) ratios were 1 and 5 mol%, and these samples were labeled as HAp_1MIX and HAp_5MIX, respectively, while non-substituted powder was labelled as HAp.
- A CAD model (Fig. 1a-d) of the desired structure that mimic natural bone tissue was designed by using nTopology 3.35.2 software.
- Obtained HAp_800, HAp_1MIX_800 and HAp_5MIX_800 powders were mixed with a commercial photocurable resin premix supplied by Lithoz GmbH in a volume % ratio of 38/62 as powder/resin.
- The cylinder shape CAD models with outer dimensions of 6 × 6 mm, in diameter and height, were fabricated using the CeraFab 7500 (Lithoz GmbH) DLP vat photopolymerization printer with a layer thickness of 25 µm.
- The debinding and sintering schedules are shown in Fig. 1e.

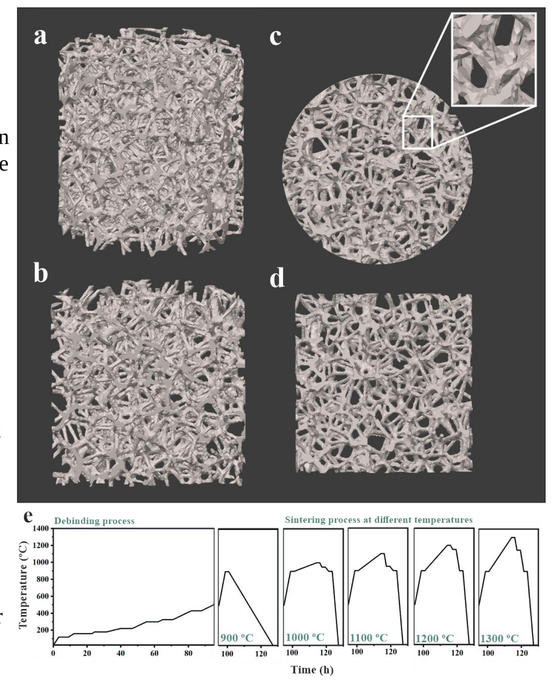

Figure 1. (a) Side and (b) cross-section views of the CAD model of scaffold. The CAD model thin cross-sections imaged from (c) the x-axis direction and, (d) z-axis direction showing the topology of the designed porosity as in inset. (e) Debinding and sintering schedule of the heat treatment process employed to obtain the final sintered products.

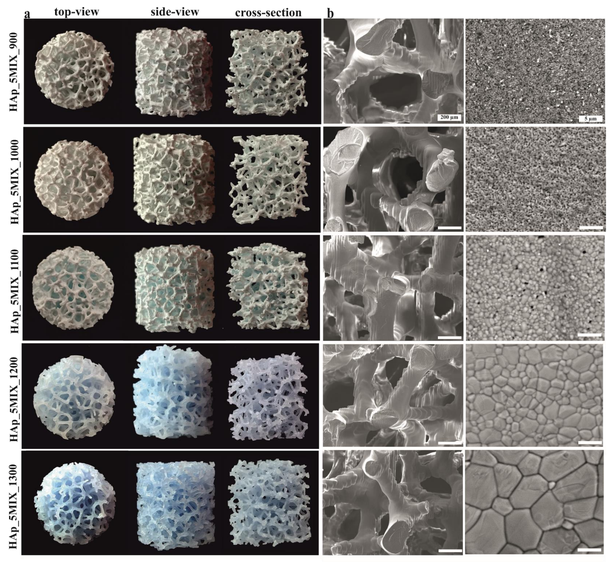

Figure 3. (a) Morphology and (b) microstructure of fabricated scaffolds (HAp_5MIX) sintered at different temperatures. Scale bar: 200 and 5 µm.

- All fabricated scaffolds exhibit highly porous structures characterized by open and interconnected pores. Uncured slurry presents difficulties in cleaning small and interconnected pores within scaffolds. Cross-sectional views (Fig. 3a) affirm the thorough cleaning of the scaffolds, despite the complexity of their structure.
- At higher magnification, the effect of sintering temperature on grain size and surface porosity become more apparent.

- The micro-CT analysis of scaffolds (Fig. 4a) was used to determine pore size distribution (Fig. 4b) and proportion of open porosity compared to the overall porosity as a function of certain sized particles that can enter the scaffold (Fig. 4c). The results of porosity, average pore size and wall thickness measurements are summarized in Table 1.
- Pores size in scaffolds ranges mostly from 10 to 900 µm. Total porosities of the scaffolds were similar with average of 76.24 ± 1.32 vol.%, mimicking the morphology of cancellous bone tissue. Similar average pore size and wall thickness were determined between the samples with average of 546.25 ± 10.95 and 217.03 ± 8.98 µm, respectively.

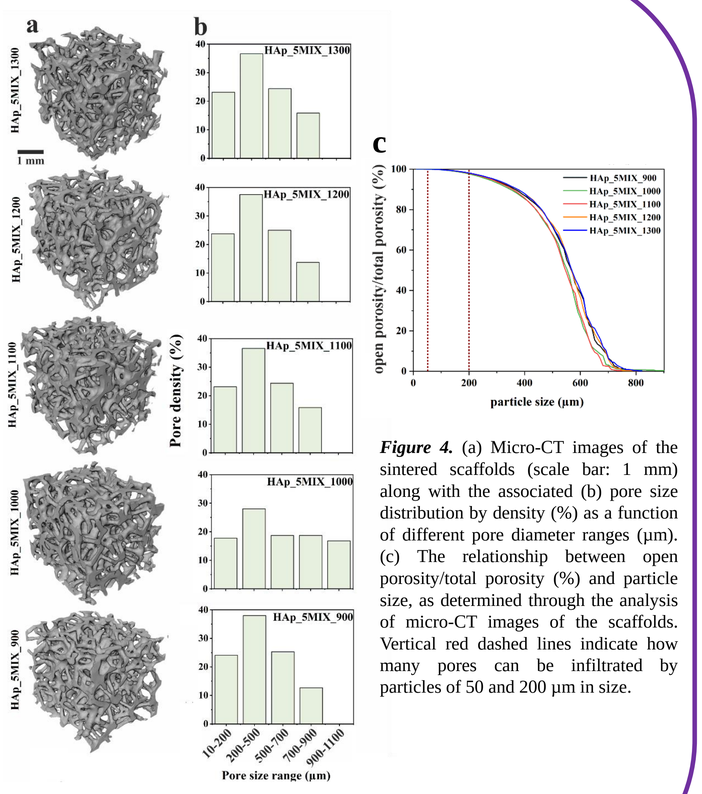

Figure 4. (a) Micro-CT images of the sintered scaffolds (scale bar: 1 mm) along with the associated (b) pore size distribution by density (%) as a function of different pore diameter ranges (µm). (c) The relationship between open porosity/total porosity (%) and particle size, as determined through the analysis of micro-CT images of the scaffolds. Vertical red dashed lines indicate how many pores can be infiltrated by particles of 50 and 200 µm in size.

Scaffold	porosity (%)	average pore size (µm)	wall thickness (µm)
HAp_5MIX_900	75.55	551.94 ± 133.55	216.16 ± 52.81
HAp_5MIX_1000	74.83	536.05 ± 135.45	214.59 ± 52.31
HAp_5MIX_1100	75.54	532.79 ± 126.94	208.24 ± 55.60
HAp_5MIX_1200	77.43	554.54 ± 131.01	201.21 ± 48.98
HAp_5MIX_1300	77.84	555.93 ± 133.41	194.93 ± 42.33

Table 1. The porosity parameters of the composite scaffolds based on micro-CT image data.

Mechanical properties of fabricated scaffolds

- Scaffolds HAp_5MIX_900 and HAp_5MIX_1000 exhibit typical stress-strain curves for porous biological scaffolds where the strength was repeatedly lost and recovered as each layer of walls collapsed. In contrast, scaffolds HAp_5MIX_1100, HAp_5MIX_1200, and HAp_5MIX_1300 exhibit linear elastic deformation followed by brittle failure of the whole structure.

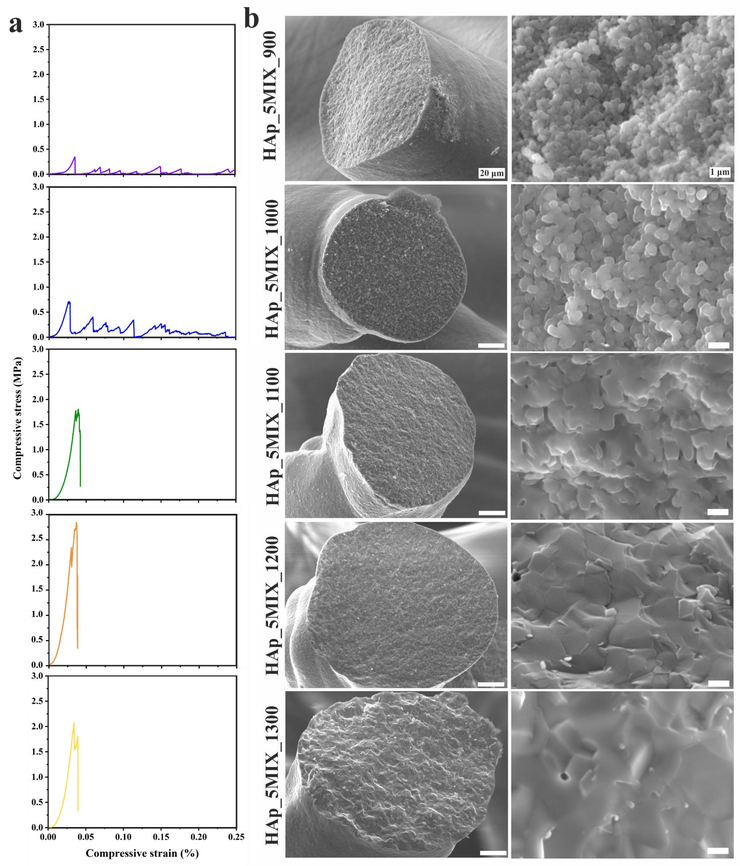

Figure 5. (a) Experimental stress-strain response. (b) Microstructures of the cracks after compressive strength analysis. Scale bar: 20 and 1 µm.

- The mechanical properties of the scaffolds sintered at 1100, 1200 and 1300 °C are in accordance with the mechanical properties typically observed in trabecular bone.

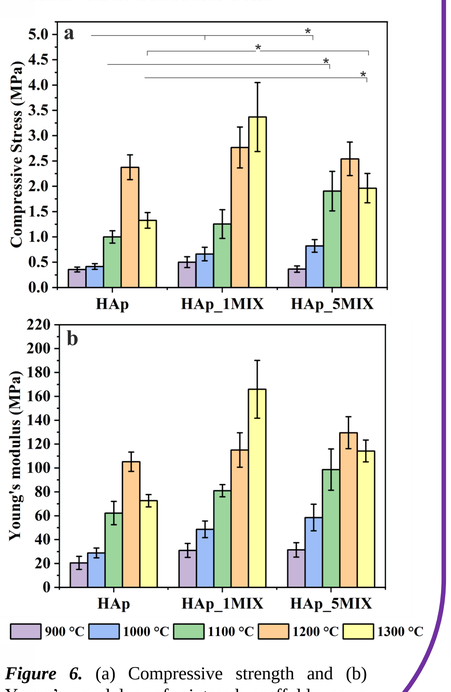

Figure 6. (a) Compressive strength and (b) Young's modulus of sintered scaffolds as a function of sintering temperature. The significant difference between two groups: * $p < 0.05$

Conclusion: This study aimed to produce biomimetic scaffolds mimicking bone microstructure and composition. Using ceramic vat photopolymerization, trabecular-like porous scaffolds from custom HAp powders substituted with Sr^{2+}, Mg^{2+}, and Zn^{2+} ions were fabricated. Scaffolds were sintered at temperatures from 900 to 1300 °C. Mechanical analysis showed properties resembling trabecular bone, with HAp_1MIX_1300 exhibiting the highest strength. Micro-CT confirmed scaffold structures resembling cancellous bone tissue. Substituted ions led to a biphasic CaP system post-sintering, potentially enhancing bioactivity. Future research will explore biological properties and printing smaller pore size ranges to address cleaning challenges.

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