# Sintered 3D-Printed Hydroxyapatite Scaffolds for Bone Regeneration

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INTRODUCTION: Bone critical size defects require surgical intervention to induce healing, where the most common revision methods utilize autologous bone grafts. Despite this being the gold standard for treatment, grafting requires multiple surgeries to get bone graft material, increasing comorbidities and likelihood of infection [1]. As an alternative, a recent study by our group demonstrated the benefits of enhancing cryogel scaffold fabrication with the addition of a plastic 3D-printed framework for bone applications [2]. Further, there are additional benefits to increasing scaffold bioactivity where previous research has demonstrated increased mineralization both *in vitro* and *in vivo* with incorporation of calcium-based minerals (e.g., hydroxyapatite). Recently, our group has conducted similar research utilizing a 3D-printed mineral structure composed of 4:1 hydroxyapatite-wollastonite. These mineral resins designed for Digital Light Processing (DLP) 3D-printing contain a binder consisting of carbon-based methacrylate oligomers and the photoinitiator diphenyl(2,4,6-trimethyl benzoyl)phosphine oxide, both necessary for curing the resin. However, these materials have been shown to negatively affect the 3D-printed lattice's density and strength and are cytotoxic. The process of sintering hydroxyapatite ($Ca_5(PO_4)_3OH$) has been demonstrated to rid the lattice of such binders, preserving pure hydroxyapatite with improved mechanical properties and crystalline structure. Utilizing a standard combustion-sintering cycle for mineral resins, we *hypothesize* that a sintered 3D-printed hydroxyapatite-wollastonite mineral lattice will have increased strength via compression tests, clear fusion of mineral components on Scanning Electron Microscopy (SEM), a reduction of the carbon-based elements from the binder quantified with Energy Dispersion X-Ray spectroscopy (EDS), and a reduction of peak intensity for hydroxyapatite and wollastonite crystals from X-ray diffraction analysis (XRD), as compared to an unsintered lattice.

METHODS: Hydroxyapatite lattices were 3D-printed on the Bison 1000 3D Printer (Tethon) using a 4:1 hydroxyapatite-wollastonite resin (Tethon Osteolite; *N*=40). Half of these scaffolds were sintered in a Thermolyne 1.3l Small Benchtop Muffle A1 furnace, at a sintering cycle of 50°C per hour to 232°C, holding 8 hours, ramping 75°C per hour to 700°C, holding 1 hour, and ramping 100°C per hour to 1050°C, holding for one hour. Both sintered and unsintered lattices were compressed to 60% strain using an Instron 5544. Scaffolds were sputter coated with gold and imaged using SEM to compare surface features (*N*=3). Finally, sintered and unsintered lattice elemental and crystalline composition were determined via energy-dispersive X-ray spectroscopy and X-Ray Crystallography (XRD) respectively.

RESULTS: SEM images for unsintered lattices display a combination of hydroxyapatite spherical particles and wollastonite ($CaSiO_3$) crystal structures bound together by the binder (Fig. 1A). Comparatively, SEM images (Fig. 1B; arrow) and EDS scans (Fig. 2) of the sintered lattice reveal early signs of the mineral structures fusing together, with EDS confirming that structures mainly consist of calcium (blue), oxygen (green), phosphorous (yellow) and silicon (pink), with trace amounts of nitrogen, chlorine, tin, and carbon. However, with the reduction of the binder after combustion and sintering, lattices exhibited a striking reduction in density and structural integrity. While the compressive modulus for the unsintered lattices were around 0.0652 ± 0.0209 MPa, sintered structures were too brittle to compress. XRD peaks confirm that the lattice maintains its primary crystalline structures after sintering, but intensity of primary crystalline peaks decreases (Fig. 3B), also suggesting that fusion of hydroxyapatite and wollastonite is occurring.

DISCUSSION: These results demonstrate that combustion and sintering is removing the cytotoxic materials and reorganizing the mineral structures of the lattice. For example, when there are fewer individual crystals, the peaks' intensity will decrease as the x-rays are less likely to be diffracted back. While this is promising for increased biocompatibility, further studies are being conducted to determine a sintering cycle that will effectively fuse the mineral structures to improve their overall physical properties. Additionally, mineralization studies are currently being conducted to compare sintered and unsintered lattices, providing additional information on their bone mineralization potential.

SIGNIFICANCE: This study aims to investigate the novel technology of 3D-printed hydroxyapatite-based lattices as a bone graft substitute to improve clinical outcomes for patients with critical defects. Optimizing the sintering outcomes of mineral based 3D-printed structures will be highly relevant for clinical application and translation potential in treating bone critical size defects.

REFERENCES: 1. Balwin P., et al. Journal of Orthopedic Trauma (2019); 2. Olevsky, L., et al. Bioengineering (2023).

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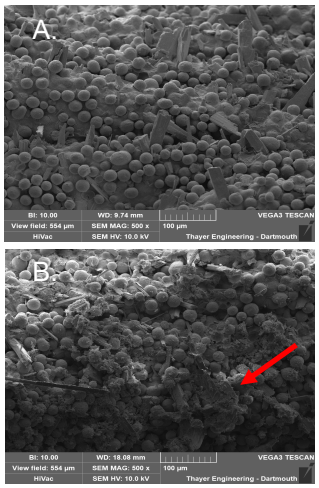


Figure 1: SEM of unsintered (top) and sintered (bottom) lattices.

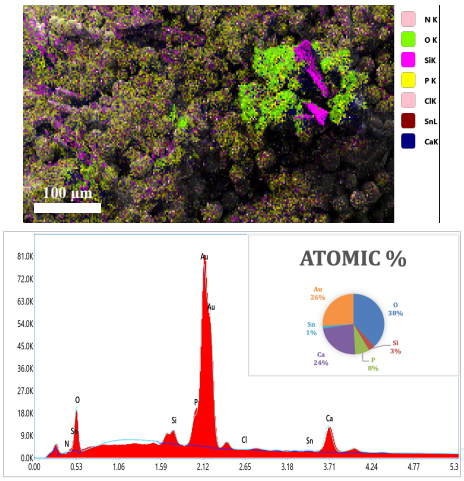


Figure 2: EDS results depicted in mapping, peak graph, and pie chart.

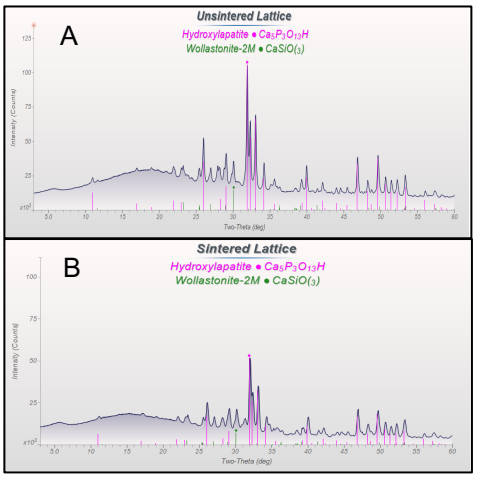


Figure 3: XRD data cross-referenced for hydroxyapatite and wollastonite via JADE software.