

Composite Bone Mineral Scaffolds for Bone Regeneration of Segmental Defects

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INTRODUCTION: A segmental bone defect is defined as a gap in bone tissue that is approximately two times larger than the diameter of the tissue. In general, a bone defect of this size—most commonly the result of acute high-energy trauma or bone tumor resection—will not heal on its own and requires surgical intervention to regain structure and function. A major challenge during reconstruction is securing bone grafts in place while providing necessary support and stability. Surgeons have adopted a novel method of reconstructing these defects with 3D printed cages. Cages, printed from titanium or polycaprolactone (PCL), are engineered to encapsulate patient bone graft material, providing a rigid structure that mimics bone shape, while enabling the graft access to nutrients and a vasculature source. However, standard bone graft materials possess limitations including donor site morbidity, reduced tissue supply (major bone loss), and potential graft rejection. These limitations support a pressing need for the development of alternatives to graft materials for bone regeneration. Tissue-engineered scaffolds have been shown to promote cellular adhesion, proliferation, and tissue regeneration at implant sites. Cryogels consist of polymer solutions cross-linked at subzero temperatures, where thawing results in a sponge-like, macroporous structure. While cryogel scaffolds are mechanically durable, they are not sufficiently stiff nor stable enough to integrate directly with metallic implants and large defects. Recently published work from our group demonstrated effective cryogel formation and integration with 3D printed plastic cages [1]. However, there was evidence of cellular death after 21 days, indicating that the plastic cage may be cytotoxic. Alternatively, while previous work has shown strong biocompatibility and mineralization potential of hydroxyapatite (HA) as an additive in cryogel scaffolds, no studies have combined 3D printed HA structures with cryogel, which has the potential to decrease the cytotoxic effect seen from plastic while increasing the mechanical integrity and bone regeneration potential of the scaffold. We hypothesize that a cryogel formed with a 3D printed HA cage will exhibit a significant increase in mechanical stiffness and mineralization potential, with improved adhesion, infiltration, and viability of osteoblast-like cells, when compared to both a plastic composite cryogel and a cryogel formed without a cage (control).

METHODS: Mineral cages—with parameters chosen from previous work [1]—were printed from HA resin (Osteolite; Tethon) on a DLP printer (Tethon). Chitosan/gelatin (1:4) solution was mixed and poured into the 3D HA cages, where crosslinking occurred at subzero temperatures. Cryogel scaffolds formed without a mineral or plastic cage served as a control. All scaffolds were imaged by scanning electron microscopy (SEM; Tescan Vega3) to quantify average pore diameter (ImageJ). Mineral composite scaffolds were scanned using energy-dispersive x-ray spectroscopy (ThermoFisher) to confirm elemental composition ($N=3$). Samples were compressed at 50% strain (Instron 5544 electronic load frame) to assess mechanical integrity ($N=3$ /group). Scaffolds were placed in water, and their masses measured over time points spanning 24 hours to assess swelling kinetics ($N=3$ /group). All scaffolds were seeded with MG-63 osteosarcoma cells and incubated for 3, 5, 14, and 21 days, fixed, sectioned and imaged with spinning-disk confocal microscope (Nikon SoRa) to assess infiltration, adhesion, and viability ($N=3$ /group/time point). Ongoing work is focused on mineralization potential, where all scaffolds will be soaked in washes of 200 mM CaCl_2 and 120 mM Na_2HPO_4 and then analyzed with SEM, ultimate compression, and alizarin red stain (ARS) for mineral formation ($N=5$ /group). One-sample t-test, two-sample t-test, and one-way ANOVA testing with Tukey post hoc were used to test significance ($P < 0.05$).

RESULTS: SEM (Fig1A,B) and EDS images depict crystalline structures and confirm the elemental makeup of the 3D printed cage to be greater than 80% calcium and phosphorus combined. The average pore diameter of the composite mineral scaffold was found to be $66 \pm 40 \mu\text{m}$, which is not significantly different compared to a control cryogel (Fig1D). Stress-strain results indicate a lower yield strength, but a higher resilience for mineral composite scaffolds as compared to the more brittle plastic composite scaffolds (Fig2A). Mineral composite scaffolds were significantly stiffer than controls, with compressive moduli values of 0.6 MPa, which were approximately 100x higher (Fig2B). Like the plastic composites, while absolute swelling was decreased compared to a control, mineral lattice composites achieve absolute swell capacity of about 300% of their dry weight in 2 minutes (Fig1C). Confocal microscope images of the surface of the composite mineral samples (FigA1,A2) depict a greater number of cells adhered when compared to a control (Fig3B) and plastic composite scaffolds (Fig3C) after 5 days of incubation.

DISCUSSION: These results demonstrate that combining cryogels with a 3D printed mineral framework results in a mechanically stable, porous structure with significantly increased compressive stiffness, while retaining relative swelling capability. Additionally, early qualitative cell studies indicate more effective surface cellular adhesion of osteoblast-like cells. With results and literature supporting that the addition of inorganic minerals in scaffolds improve osteogenic activity and increase mineralization rate, ongoing studies are investigating long term cellular interactions with the mineral composite scaffolds.

SIGNIFICANCE: This innovative technology has the potential to be applied to patient-specific bone reconstruction in orthopedic and plastic surgery, especially long bone reconstruction. The work in this proposal will lay the foundation for a technology that, with further animal studies and clinical development, can replace traditional bone grafts and improve surgical outcomes in large-scale bone reconstruction.

REFERENCES: 1. Olevsky, L., et al. *Bioengineering* (2023)

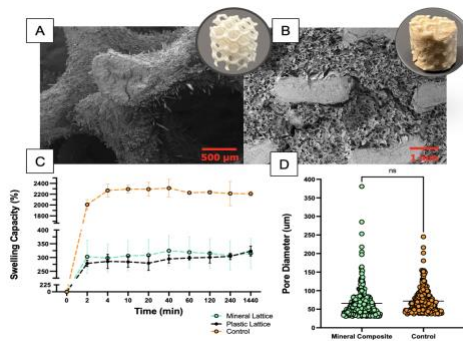


Figure 1: (A) SEM of printed mineral cage and (B) mineral composite scaffold; (C) Swell capacity values; (D) Pore size measurements

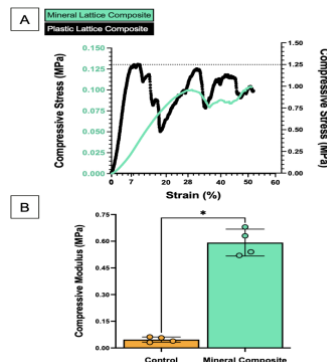


Figure 2: (A) Stress-strain curves; (B) Young's Modulus comparison

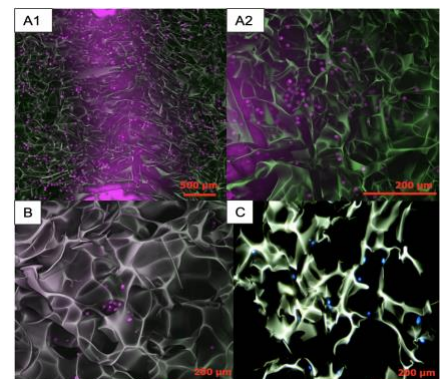


Figure 3: Confocal images of (A1,A2) composite mineral scaffold, (B) control, (C) plastic composite scaffold