# Final Report

**Patient-Specific Three-dimensional (3D) Printed Bioceramic Scaffold for Alveolar Ridge Preservation**

Condensed Summary* Over the funding period, we have been able to successfully demonstrate the development of a novel bioceramic ink for 3D printing of personalized and defect-specific scaffolds for bone regeneration. In detail, during the time of the award, my lab in conjunction with Dr. Bhaduri's (Co-Investigator) research team at University of Toledo (OH), as well as collaborators from the University of Michigan School of Dentistry (Dr. Darnell Kaigler) and at the University of Utrecht (The Netherlands, Dr. Jos Malda) have made significant strides in identifying the role of amorphous magnesium phosphate as both a novel bioceramic with significant osteopromoting properties as well as the base material for the proposed and clinical promising ceramic ink for developing personalized scaffolds for bone regeneration. Here, I will summarize major accomplishments related to the proposed project, which, collectively, have allowed the successful acquisition of a large 5-year R01 grant from NIDCR/NIH (July 2022 – June 2027) to pursue further work to expedite the clinical translation of the proposed innovation.

## Aims and Outcomes*

**AIM 1. To synthesize the AMP ink and develop 3D printed scaffolds.** [100% completed].

Magnesium phosphates are considered a better alternative to traditional calcium phosphates (e.g., hydroxyapatite, HAp). These materials have sufficient solubility under *in vivo* conditions, while $Mg^{2+}$ is a potent inhibitor of HAp crystal growth. This fact helps retain the bioactivity of Mg-phosphates. Indeed, amorphous magnesium phosphate (AMP) has been found to accelerate the differentiation and mineralization of pre-osteoblasts compared to its crystalline counterpart. We hypothesized that by using our novel AMP particles with defined morphology/composition, we would be able to synthesize a bioceramic ink aimed at developing defect-specific scaffolds. The first step in the development of the AMP ink was the successful demonstration of the synthesis of the proposed AMP particles. As an initial study to determine the therapeutic potential of AMP in positively influencing mesenchymal stem cells (MSCs) differentiation into osteogenic lineage, we reported on (1) the synthesis of a bone tissue-specific bioink consisting of an extracellular matrix (ECM)-derived hydrogel and AMP particles (**study published – reference #1**) and on (2) the fabrication of a fiber-reinforced hydrogel-based AMP-containing membrane for guided bone regeneration applications (**study published – reference #2**). Briefly, *in vitro* cellular responses (cell-laden biofabricated constructs), including mineralization (data not shown) and osteogenic gene expression **(Fig. 1)**, were evaluated by encapsulating human-derived dental pulp stem cells (DPSCs) – cultured in basal medium. Our findings demonstrated that AMP incorporation into a biocompatible hydrogel can trigger the osteogenic differentiation of encapsulated DPSCs without any additional use of growth factors and stimulate *in vivo* bone regeneration (data not shown).[1]

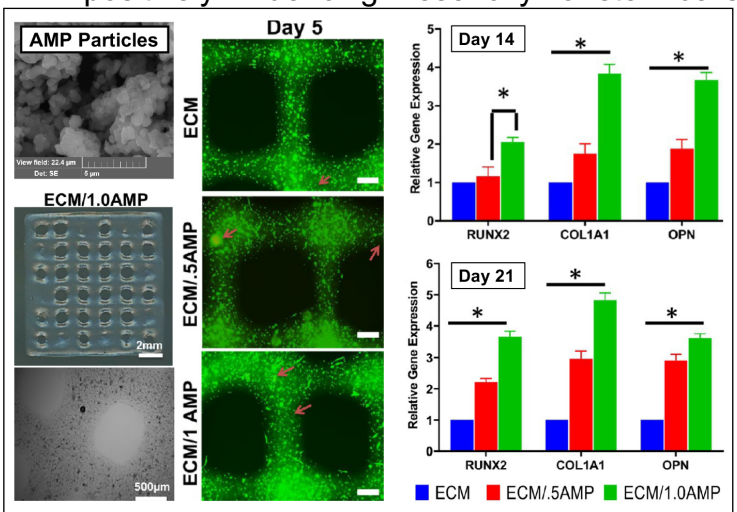


**Fig. 1. (Left)** SEM image of AMP particles and 3D printed constructs without cells – shape fidelity analysis. **(Middle)** Calcein AM (green) and PI (red) staining assay for live/dead analysis within cell-laden bioprinted constructs after 1, 3, and 5 days. DPSCs show an elongated morphology in AMP-modified constructs. **(Right)** Enhanced expression of osteogenic-genes within bioprinted constructs when compared to unmodified (ECM) hydrogel (n=4).[1]

Relevant to the fabrication of the AMP ink, next, we hypothesized that by using our novel AMP particles and in combination with other specific reagents, such as polyvinyl alcohol (PVA) – to control the hydrolysis of AMP into a crystalline phase as well as citric acid and tween-80 – to delay initial setting, we could ultimately develop an AMP-based paste for 3D printing of personalized bone scaffolds.

Worth mentioning, after noting the nearly immediate degradation of the above-referenced scaffolds upon immersion in PBS **(Fig. 2)**, we added *suberic acid* in the PVA preparation.[50] AMP scaffolds were fabricated for biological (*in vitro* and *in vivo*) evaluations. Although the compressive modulus of the printed AMP scaffolds was lower than the HAp/α-TCP control **(Fig. 2),** it is in agreement with similarly porous 3D printed scaffolds (HAp 83 MPa and TCP 37 MPa). Indeed, the modulus of our scaffolds exceeds some of the values commonly reported for trabecular bone (0.5-14.6 MPa). After initial cytocompatibility screening, no significant differences were found between AMP and HAp/α-TCP (data not shown); we hypothesized that the printed AMP scaffolds would support osteogenic differentiation of human-derived alveolar bone mesenchymal stem cells. As shown in **Fig. 3**, both ceramic scaffolds led to increased expression of bone-related genes compared to the control (cells on the tissue culture plate). Next, based on our promising findings, we continued with scaffold implantation using a well-established rat calvarial critical-size (ϕ=8 mm) defect model to determine both the regenerative efficacy and degradation pattern of the scaffolds **(Fig. 4)**. μCT and histological analyses (Masson's trichrome, MT) showed enhanced bone formation after 4- and 8-weeks post-implantation in defects treated with the AMP scaffolds when compared to the control (sham). Importantly, although there was histological evidence of mineralized tissue formation within defects treated with the HAp/α-TCP scaffolds, the μCT data was not included, as the degradation profile of those scaffolds was minimal when compared to the developed AMP.

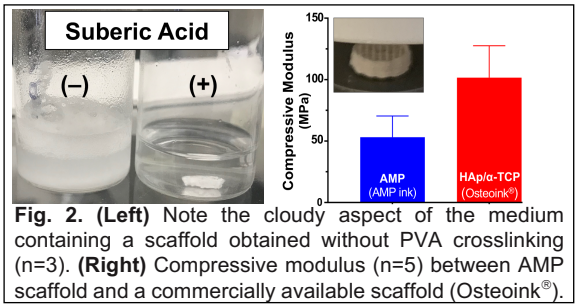


**Fig. 2. (Left)** Note the cloudy aspect of the medium containing a scaffold obtained without PVA crosslinking (n=3). **(Right)** Compressive modulus (n=5) between AMP scaffold and a commercially available scaffold (Osteoink®).

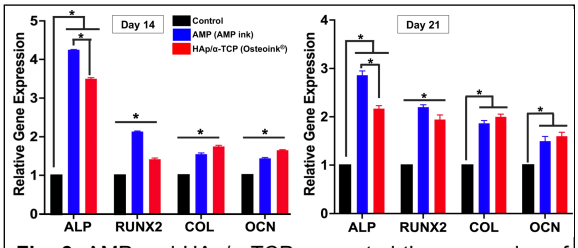


**Fig. 3.** AMP and HAp/α-TCP supported the expression of osteogenic-genes (n=4). **(Left)** Day 14 and **(Right)** Day 21.

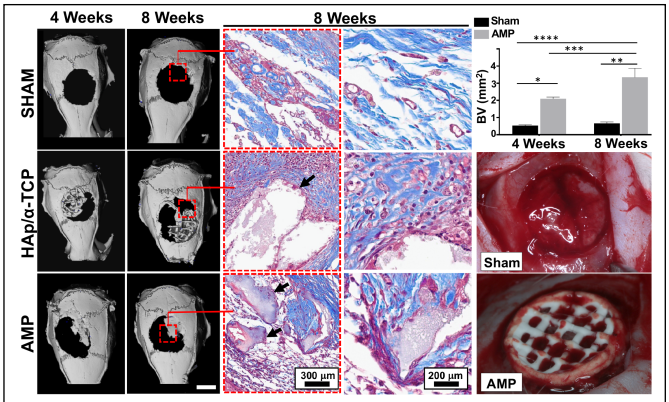


**Fig. 4.** μCT reconstructions after 4- and 8-weeks post-surgery. Red squares in the μCT images represent areas shown in the MT-stained sections. Black arrows (scaffold remnants). Higher magnification images show the tissue structure and cellular organization supporting mineralized tissue formation. μCT data not included for the HAp/α-TCP group due to substantial scaffold presence (n=4).

**AIM 2** *To determine the regenerative capacity of the 3DP AMP-based scaffolds using a well-established rat model of alveolar ridge preservation.* **[65% completed]**.

Prior to implanting the personalized 3DP AMP-based scaffolds in the rat model of alveolar ridge preservation, our group had the opportunity to pursue a pilot study (in the dog) using a highly translational animal model of guided bone regeneration to determine the regenerative capacity of our bioceramic scaffolds in a block-like shape. **Figure 5** shows the details of the surgical procedures and implanted scaffolds along with preliminary data (cone beam CT) of the regenerated sites. Unfortunately, we had issues with the decalcification of the bone/scaffold samples, and we hope to publish these findings later this year. We are currently working on the printing of the personalized/root-like scaffolds to be implanted in the rat model in the Fall 2023.

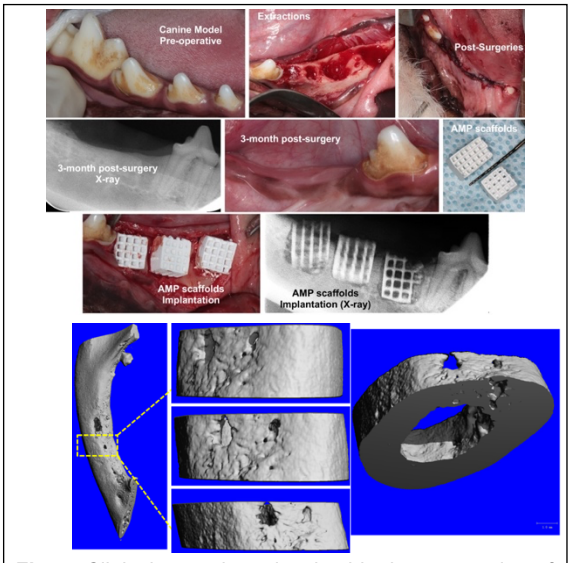


**Fig. 5.** Clinical procedures involved in the preparation of the bone defects in the mandible and 3D printed AMP scaffolds. MicroCT reconstructions after 8-weeks post-surgery. From a qualitative perspective, it can be stated that the defects were able to heal nearly 80%. (n=3).

## Final Conclusions and other supporting documents

Our findings shows that a novel biocompatible and highly osteogenic biomaterial, i.e., amorphous magnesium phosphate (AMP) can be used as a bioceramic ink

for 3D printing of clinical scale and personalized scaffolds for alveolar ridge preservation. Collectively, emerging digital technologies including CBCT imaging, 3D surgical planning, and (bio)printing can be integrated to address this unmet clinical challenge.

## Plans for Publication*

The work related to the exploitation of AMP as the major component of a bioceramic ink for the fabrication of personalized scaffolds for bone regeneration as well as a chemical modifier of hydrogels to promote bone formation in craniomaxillofacial defects led to numerous publications in high-impact Journals as well as to major NIH funding (details of the publications are listed below). The findings of the proposed rat model (AIM 2) should be finalized and written by Dec 2023 and submitted for publication Winter 2024.

List of publications related to the OsteoScience funding support.


1. Dubey N, Ferreira JA, Malda J, Bhaduri SB, Bottino MC. Extracellular Matrix/Amorphous Magnesium Phosphate Bioink for 3D Bioprinting of Craniomaxillofacial Bone Tissue. ACS Appl Mater Interfaces. 2020 May 27;12(21):23752-23763. doi: 10.1021/acsami.0c05311.
2. Dubey N, Ferreira JA, Daghrery A, Aytac Z, Malda J, Bhaduri SB, Bottino MC. Highly tunable bioactive fiber-reinforced hydrogel for guided bone regeneration. Acta Biomater. 2020 Sep 1;113:164-176. doi: 10.1016/j.actbio.2020.06.011.
3. Sikder P, Ferreira JA, Fakhrabadi EA, Kantorski KZ, Liberatore MW, Bottino MC, Bhaduri SB. Bioactive amorphous magnesium phosphate-polyetheretherketone composite filaments for 3D printing. Dent Mater. 2020 Jul;36(7):865-883. doi: 10.1016/j.dental.2020.04.008.
4. Daghrery A, Ferreira JA, Xu J, Golafshan N, Kaigler D, Bhaduri SB, Malda J, Castilho M, Bottino MC. Tissue-specific melt electrowritten polymeric scaffolds for coordinated regeneration of soft and hard periodontal tissues. Bioact Mater. 2022 Apr 22;19:268-281. doi: 10.1016/j.bioactmat.2022.04.013
5. Daghrery A, Ferreira JA, de Souza Araújo IJ, Clarkson BH, Eckert GJ, Bhaduri SB, Malda J, Bottino MC. A Highly Ordered, Nanostructured Fluorinated CaP-Coated Melt Electrowritten Scaffold for Periodontal Tissue Regeneration. Adv Healthc Mater. 2021 Nov;10(21):e2101152. doi: 10.1002/adhm.202101152.
6. Anderson M, Dubey N, Bogie K, Cao C, Li J, Lerchbacker J, Mendonça G, Kauffmann F, Bottino MC, Kaigler D. Three-dimensional printing of clinical scale and personalized calcium phosphate scaffolds for alveolar bone reconstruction. Dent Mater. 2022 Mar;38(3):529-539. doi: 10.1016/j.dental.2021.12.141.
7. Aytac Z, Dubey N, Daghrery A, Ferreira JA, de Souza Araújo IJ, Castilho M, Malda J, Bottino MC. Innovations in Craniofacial Bone and Periodontal Tissue Engineering - From Electrospinning to Converged Biofabrication. Int Mater Rev. 2022;67(4):347-384. doi: 10.1080/09506608.2021.1946236.
8. Daghrery A, de Souza Araújo IJ, Castilho M, Malda J, Bottino MC. Unveiling the potential of melt electrowriting in regenerative dental medicine. Acta Biomater. 2022 Jan 10:S1742-7061(22)00010-1. doi: 10.1016/j.actbio.2022.01.010.