3D Printing of Biopolymer Composites Fabricated from Polylactic Acid & Eggshell-Derived Hydroxyapatite

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Undergraduate Research Program for Honors Students 2020-2021

TITLE V STEM
POLYTECHNIC UNIVERSITY OF PUERTO RICO • SAN JUAN
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P031C160141

ABSTRACT

Hydroxyapatite (HAp) is a naturally occurring mineral form of calcium apatite, which is found in 65-70% of human bones. Waste-derived HAp is attracting considerable attention due to its excellent biocompatibility and capacity to enhance the proliferation of osteoblastic cells.

In the present work, HAp was synthesized from waste chicken eggshell and subsequently incorporated as a filler (at different concentrations) into a polymer matrix of polylactic acid (PLA) to fabricate composite filaments for fused deposition modeling (FDM) 3D printing. The filaments were fabricated via extrusion using a solvent-free process. Then, these were placed in an Ender 3-pro FDM 3D-printer to create PLA/HAp composite specimens and scaffolds.

Results from the tensile test analysis suggest that the elastic modulus of the 3D printed samples increases with the HAp content in the range from 0 - 3 wt%. Similarly, the reprocessing (re-extrusion) of the filaments, results in stiffer specimens. The impedance spectroscopy analysis suggests that the presence of HAp into the PLA polymer matrix, resulted in a composite material with enhanced conductivity.

INTRODUCTION

In the food industry, approximately 250,000 tons of chicken eggshell waste is produced annually worldwide.[1] Eggshell is mostly sent to landfills with a high management cost and additionally, it causes an environmental problem with methane production.[2]

Due to biological nature, eggshells could be a valuable precursor to obtain filler materials for the fabrication of bone substitutes or tissues scaffolds.[1] The term scaffold is used for three-dimensional (3D) biomaterial that provides a suitable environment for cells to regenerate tissues and organs. An appropriate scaffold must be capable to repair body tissues with minimum requirements, for cell growth, vascularization, proliferation, and host integration. [3]

The use of 3D printing offers the unique opportunity to manufacture scaffolds with the desired size and structure. Typically, most scaffolds consist of natural materials, proteins, inorganic materials or synthetic polymers like PLA. [4]

Over the last decade, the scientific community has been exploring the possibility to fabricate polymer composite scaffolds from waste-derived sources to reduce the cost of treatment in bone repair or replacement. However, it is still necessary to develop environmentally and economically feasible methods to fabricate this kind of structures.[1]

Figure 1. Organic waste

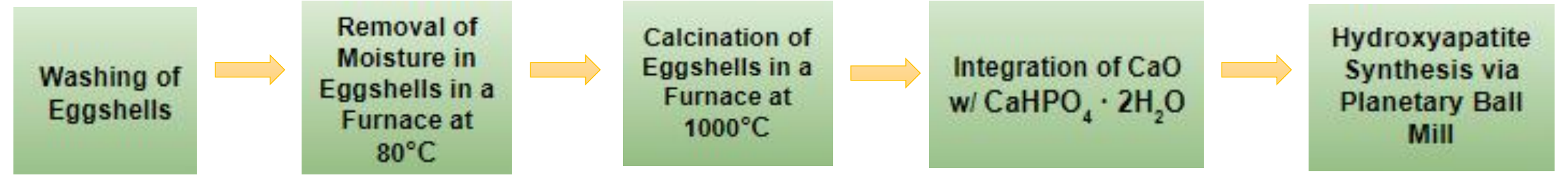
Figure 2. Eggshells

Figure 3. 3D printed scaffolds for bone tissue regeneration [3]

OBJECTIVES

This research project has four main objectives: **(1)** fabricate and characterize HAp from eggshell waste, **(2)** stablish the process conditions to fabricate PLA/HAp composite filaments having different HAp compositions using a solvent-free method, **(3)** study the effect of HAp content and extrusion reprocessing on the mechanical/electrical properties of the fabricated specimens, and **(4)** fabricate scaffolds via 3D printing using the fabricated HAp/PLA composite filaments.

METHODOLOGY



Washing of Eggshells → Removal of Moisture in Eggshells in a Furnace at 80°C → Calcination of Eggshells in a Furnace at 1000°C → Integration of CaO w/ $CaHPO_4 \cdot 2H_2O$ → Hydroxyapatite Synthesis via Planetary Ball Mill

Figure 4. HAp fabrication process

The expected reaction is:

$$4CaO_{(s)} + 6CaHPO_4 * 2H_2O_{(aq)} \rightarrow \underbrace{Ca_{10}(PO_4)_6(OH)_{2(s)}}_{\text{HAp}} + 14H_2O_{(l)}$$

Figure 5. Furnace

Figure 6. eggshells grinding

Figure 7. Eggshells calcined at 1000 °C (CaO)

Figure 8. High energy planetary ball mill

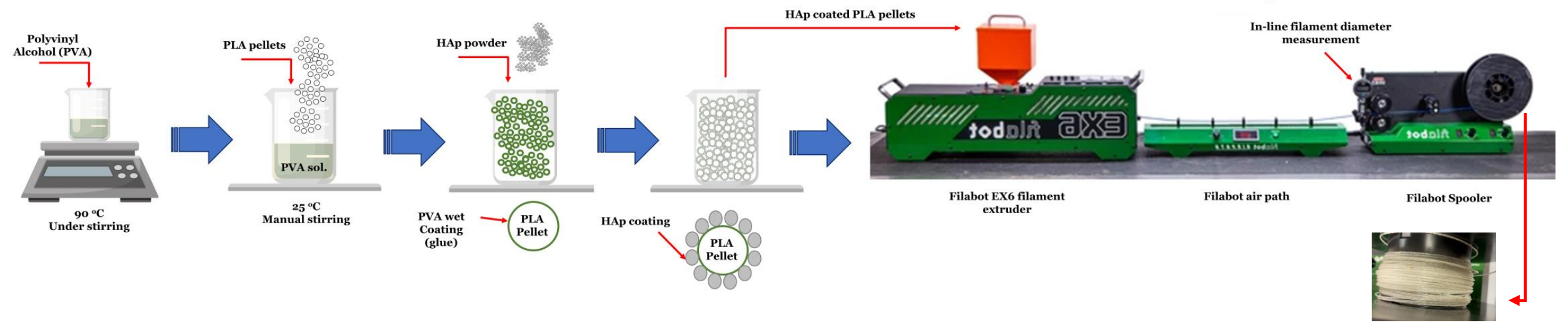


Figure 9. Filament fabrication process

The temperatures of the extruder chamber zones were varied to fabricate each filament. To reprocess the filaments, these were cut in small pieces and fed to the extruder hopper to fabricate a new filament.

The specimens and scaffolds were fabricated using a fused modeling deposition (FDM) 3D printer from Creality® equipped with a 0.2-mm, 0.4-mm, or 1-mm nozzle set at 200°C, while the plate temperature was set at 60°C. The speed chosen for the 3D printer was 30 mm/s with a line pattern.

To determine the electrical properties of the HAp/PLA specimens, a Keysight 16451B dielectric test fixture connected to a high-resolution E4990A impedance analyzer (IA) was be used. The conductivity values were determined at 20,000 Hz.

To determine the mechanical properties of the HAp/PLA specimens, tensile tests were carried out using the ADMET tensile test machine following a modified ASTM D638-14 standards.

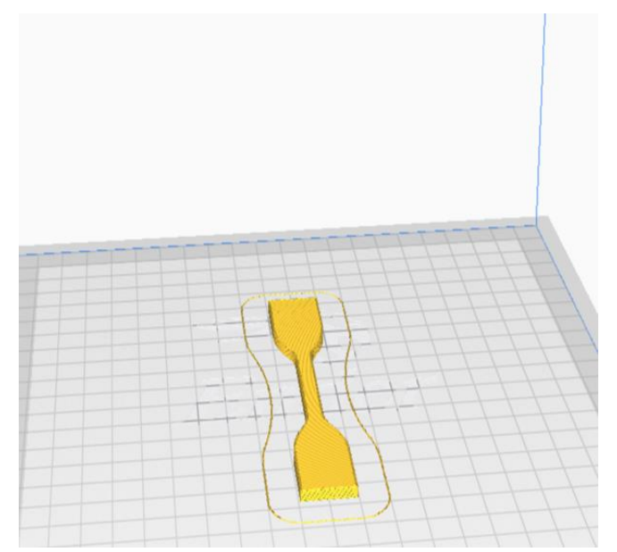


Figure 10. Ultimaker Cura software showing the created model

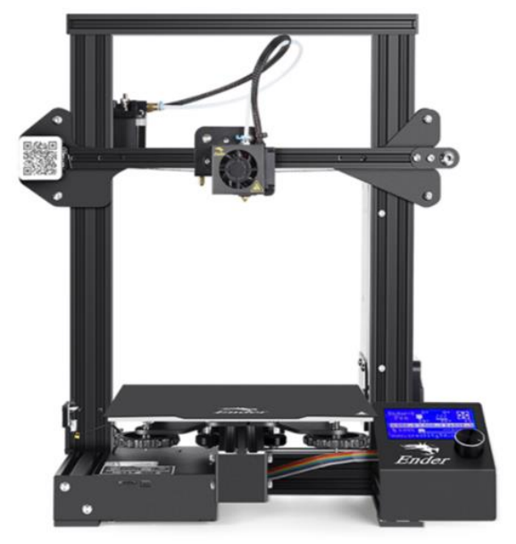


Figure 11. Ender-3 Pro 3D Printer from Creality®

RESULTS

Fabricated Materials and Specimens

Figure 12. Fabricated HAp

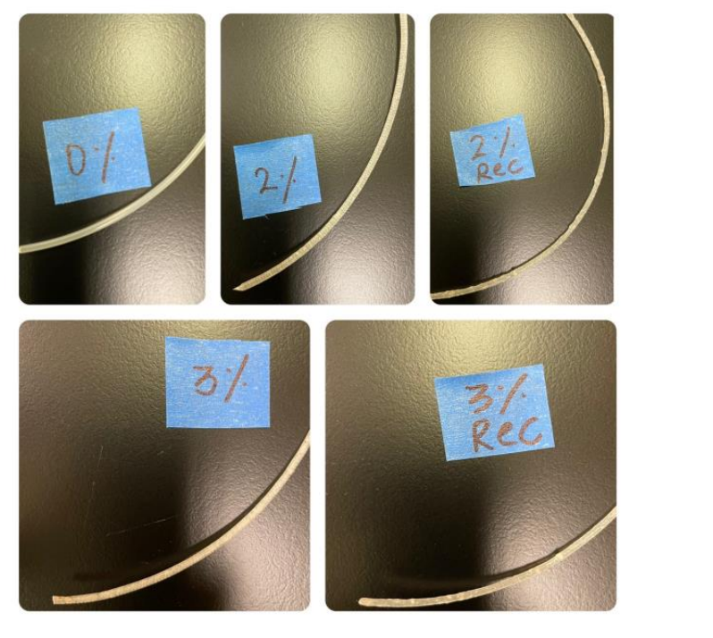


Figure 13. HAp/PLA fabricated filaments

Filament thickness: 1.6 - 1.8 mm
It was not possible to fabricate filaments at HAp contents of 4 wt% because the material was so brittle.

Table 1. Temperatures for the fabrication of different filaments (determined by trial and error)

Filabot chamber zone	Temperature (°C)		
	Filament with 0 wt% of HAp	Filament with 2 wt% of HAp	Filament with 3 wt% of HAp
I	160	160	160
II	170	180	180
III	120	170	170
IV	40	40	40

Important: extruder nozzle is located at the end of zone I, while the extruder hopper is located at the beginning of zone IV.

Figure 14. 3%/97%-HAp/PLA 3D printed Type I specimens

Figure 15. 3%/97%-HAp/PLA 3D printed Type II specimens

Figure 16. 3%/97%-HAp/PLA scaffold (2.5 cm diameter)

Important: All the specimens and scaffolds were fabricated using nozzles with a diameter of 1 mm, since at smaller diameters (0.2 and 0.4 mm), the fabricated filaments clog the nozzle aperture avoiding the required material flow.

HAp Materials Characterization

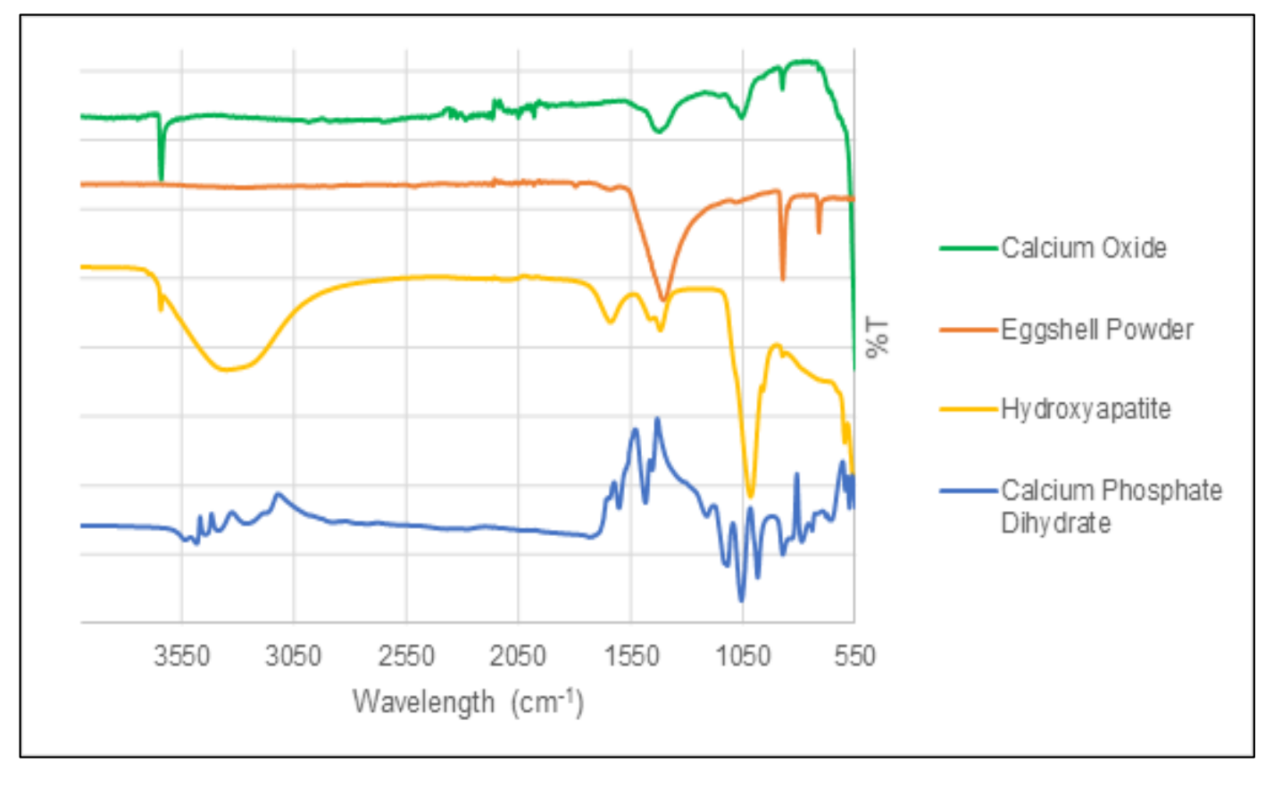


Figure 17. FTIR spectra of HAp and its precursors

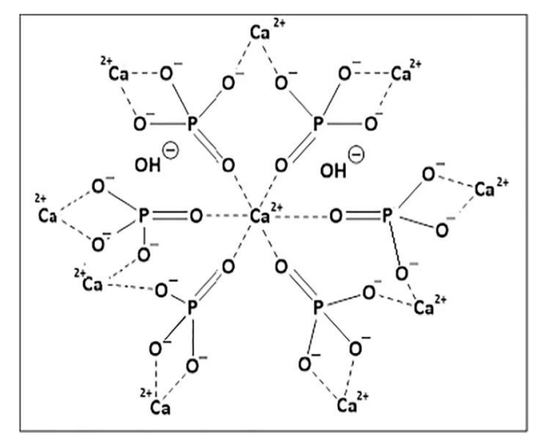


Figure 18. Chemical structure of HAp

Table 2. HAp FTIR spectrum analysis

HAp	
Wavelength peak (cm^{-1})	Corresponding functional groups
3644	Hydroxyl group stretching (OH^-)
3367 - 1628	Water bending & stretching (H_2O
1434 – 1412 & 879	Impurity: Carbonate group stretching $(CO_3)^{2-}$
1023 - 965	Phosphate group stretching (P-O)
574 - 560	Phosphate group bending (O-P-O)

RESULTS (Cont.)

Mechanical Characterization of 3D Printed Composite Samples

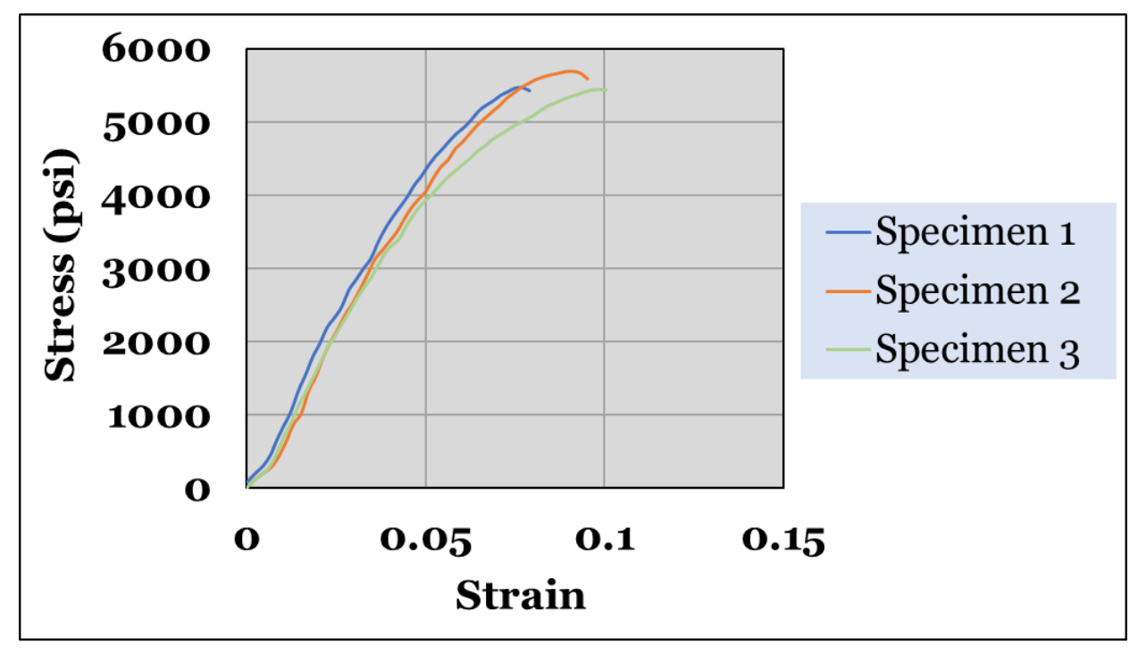


Figure 19. Stress-strain curves of 3%/97% HAp/PLA type I specimens

Figure 20. Tensile test machine with sandpaper attached to the grip surface to avoid slippage

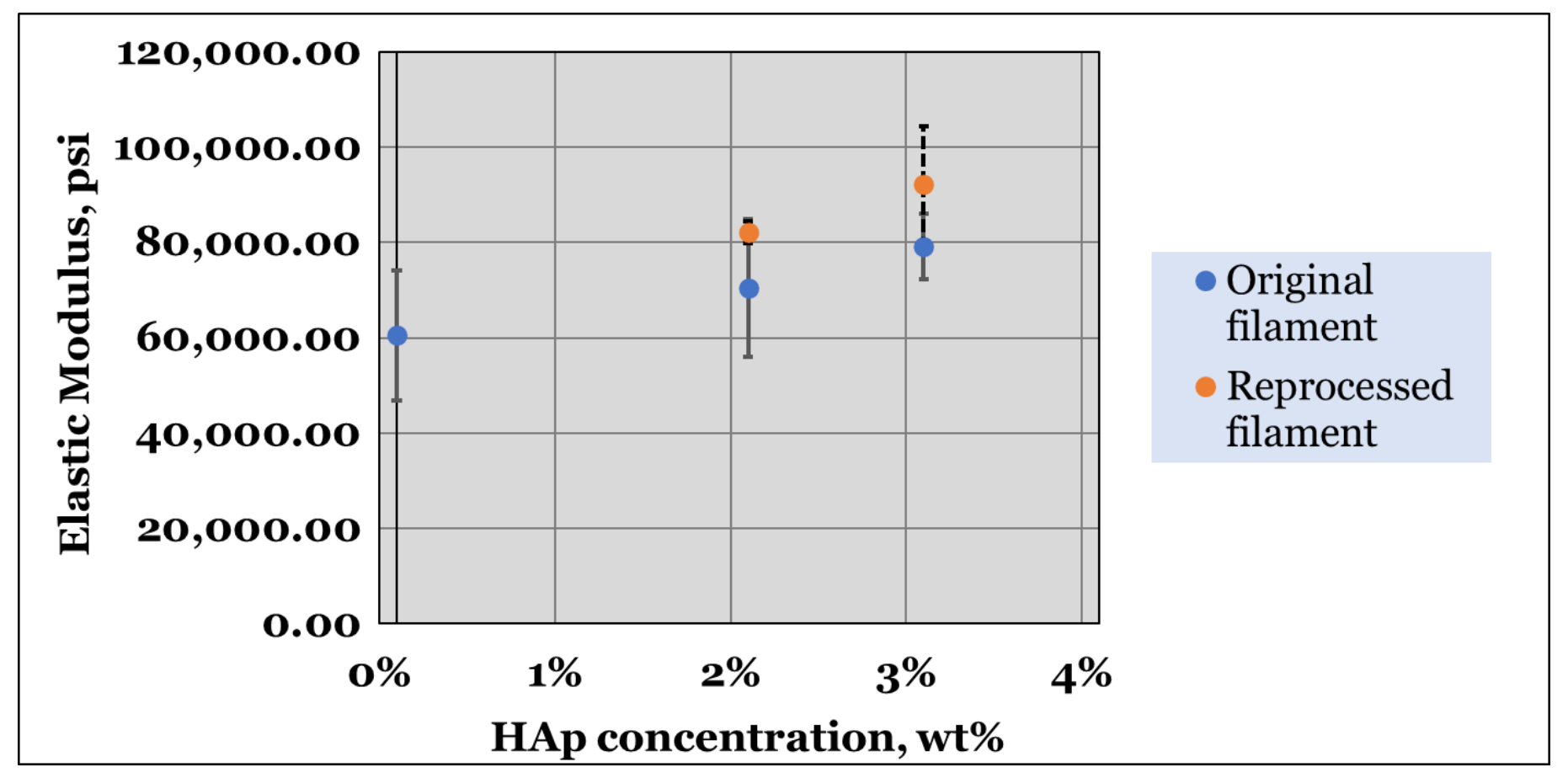


Figure 21. Elastic modulus of the type I specimens at different wt% of HAp

Electrical Characterization of 3D Printed Composite Samples

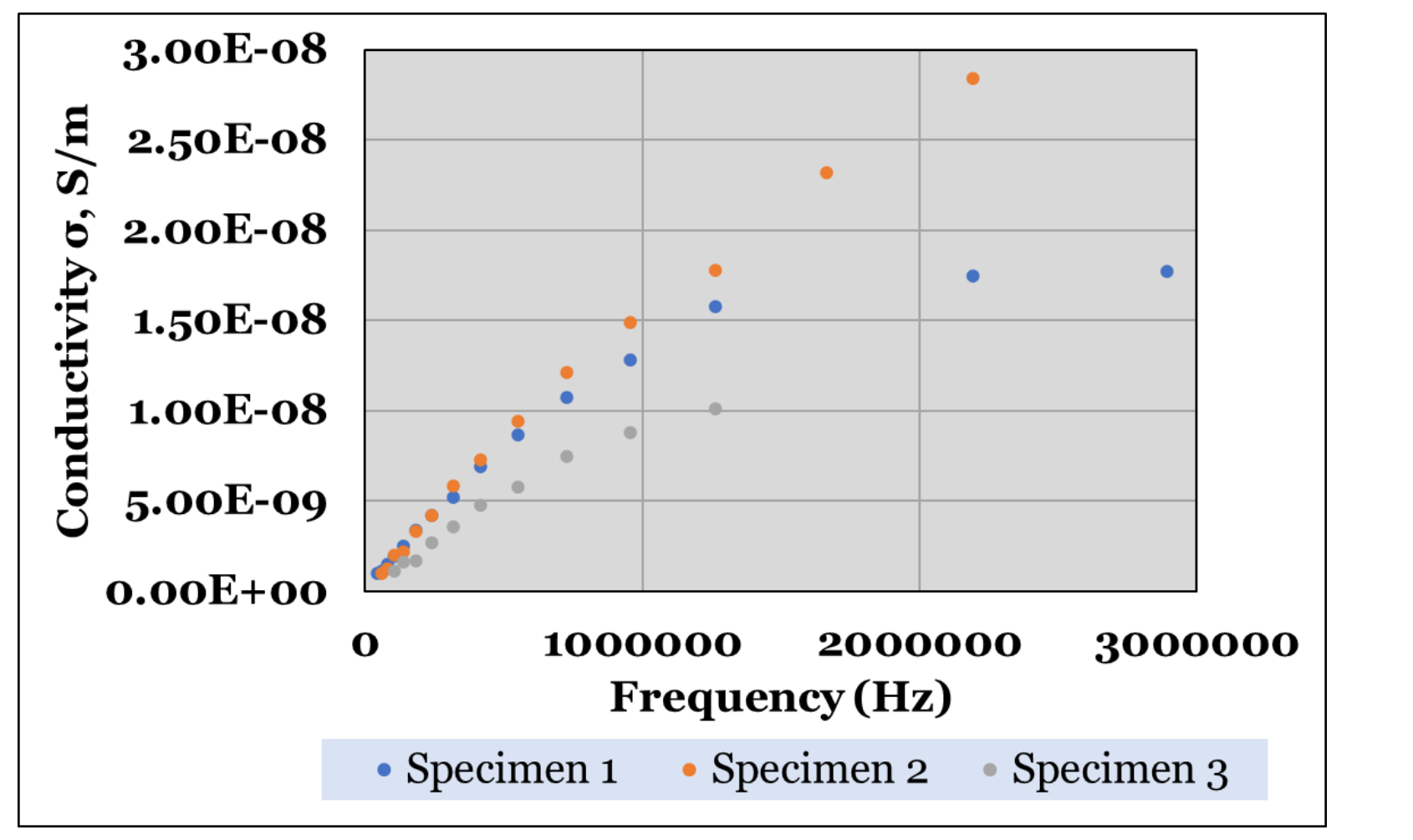


Figure 22. Conductivity-frequency of type II specimens with 2 wt% of HAp

Table 3. Type II specimen conductivity at different HAp

HAp wt%	Conductivity (S/m)
0	0.0000350 ± 4.5 E-10
2	0.0000500 ± 7.29 E-9
2 Reprocessed	0.0000600 ± 6.00 E-9
3	0.0000700 ± 7.5 E-9
3 Reprocessed	0.0000700 ± 3.5 E-10

ONGOING & FUTURE WORK

- Complete the installation and training of the new equipment: biosafety cabinet, cryogenic tank, and the fluorescent microscope to proceed with the cell culture and the biological characterization of the scaffolds.
- Study the effect of the scaffold HAp content on the osteoblastic cell's growth (work in collaboration with Prof. Maria Garriga).
- Study the microstructure of the fabricated specimens via Scanning Electron Microscopy (SEM) to understand their mechanical, electrical, and biological performance.
- Study the effect of the 3D priting orientation on the materials properties of the fabricated specimens.

Figure 23. Equipment to grow osteoblastic cells on the fabricated scaffolds at PUPR

CONCLUSIONS

- HAp was successfully fabricated from waste eggshell via calcination and subsequently, dry ball milling.
- PLA/HAp filaments having compositions of HAp ≤ 3 wt% were fabricated via a solvent-free preparation method and using a Filabot Extruder EX6. At a composition of 4 wt% of HAp, the filament was so brittle to be placed in the spooler.
- After integrating HAp fillers into the PLA, the composite materials (filament) required higher temperatures of extrusion. Selection of inappropriate temperatures caused clogging of the extruder nozzle.
- Due to the presence of clusters of HAp into the filaments, it was impossible to print specimens with nozzles having aperture diameters < 1 mm. This could be an issue for the fabrication of scaffolds with micropatterns.
- Tensile test analysis suggests that the elastic modulus of the 3D printed specimens increases with the HAp content. Also, the reprocessing (re-extrusion) of the fabricated filaments increased the elastic module of the materials.
- In the case of the impedance analysis, the results indicate that the presence of HAp into the PLA matrix increases its conductivity. This fact is relevant, since previous reports indicate that application of current or electric fields accelerate the proliferation of osteoblastic cells in tissues.

RECOMMENDATIONS

- It is recommended to sieve the hydroxyapatite after its synthesis in order to use only particle size at meso and nano scale. Employing smalls particles could increase the mechanical stability of the filaments and the 3D-printed specimens.
- Since the solvent-free method limits the amount of HAp to be used (probably because of its poor dispersion in the PLA), it is also recommended to dissolvent the PLA before mixing it with the filler. It is also recommended to use an industrial grinder and pelletizer to fabricate the pellets as part of the solvent-based mixing method.
- The use of more sophisticated FDM 3D-printers could increase the quality of the specimens.
- It is also recommended to stablish a post-treatment process for the fabricated scaffolds in order to increase the amount of hydroxyapatite exposed on the scaffold surface. It is hypothesized that the larger the amount of HAp in the surface of the scaffold, the better the osteoblastic cells growth.

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ACKNOWLEDGMENTS

We would like to thank:

- URP-HS SAFRA II program, for providing the required funding.
- Dr. Wilfredo Fariñas for allowing us to use the impedance analyzer at his laboratory.
- Prof. Maria Garriga for her contribution in the selection of the biological equipment.