From Bio-Waste to Bone Substitute: 3D Printing-Assisted Fabrication of Scaffolds Containing High Concentrations of Eggshell-Derived Hydroxyapatite

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ABSTRACT

Autografts are the gold standard for bone repair interventions due to their osteoinductive and osteoconductive properties. Unfortunately, this technique entails important drawbacks such as limited availability and donor-site morbidity. Over the last few years, the scientific community has been developing different synthetic scaffolds as an alternative to autografts. Despite the progress made in the field, there are still no ideal scaffolds with simultaneously good biocompatibility, porous three-dimensional structures, bone conduction, osteoinduction, and osteogenesis. Moreover, many of the proposed methods for the fabrication of scaffolds are difficult to customize to meet patient-specific scaffold geometry. Therefore, the present work aimed to incorporate additive manufacturing (AM or 3D printing) in the fabrication of scaffolds containing high concentrations of eggshell-derived hydroxyapatite (up to 60wt%). The goal was to mimic the hard tissue composition of the human skeleton without affecting the mechanical stability of the scaffolds.

In order to achieve the main goal, hydroxyapatite (HAp) was synthesized from waste eggshells via dry chemistry using ball milling. In addition, porous sacrificial templates were fabricated via fused deposition modeling (FDM) 3D printing, which were subsequently filled with liquid/solid dispersions containing HAp and acrylonitrile butadiene styrene (ABS) at different compositions. After drying overnight, the sacrificial template was dissolved in water to finally obtain the scaffolds. The fabricated materials were characterized via FTIR, microscopy, and compression test.

The most relevant results indicate that it was possible to use the proposed method to fabricate scaffolds with high content of hydroxyapatite (up to 60 wt% of HAp). However, the high viscosity of the slurry containing 60 wt% HAp created difficulties to fill properly the molds, resulting in fragile structures. It was also observed that the compression modulus of the fabricated scaffolds increased with the presence of HAp into the ABS polymer matrix, obtaining the maximum value at 30 wt% of HAp.

INTRODUCTION & BACKGROUND

Clinical Need

There are more than 2 million bone grafting procedures performed annually in the US alone. Despite significant progress, the repair of large segmental bone defects is still a clinical challenge which requires the fabrication of artificial bone substitutes or grafts. The available biomaterials lack the adequate mechanical strength, porosity, and/or chemical composition to facilitate cell in-growth and vascularization during bone tissue regeneration.[1]

3D Printing in Tissue Engineering

3D printing has attracted considerable attention in tissue engineering for two main reasons: (1) its versatility, ease of use, and precise control of the fabrication process, and (2) the products can be customized in shape and structure, possessing unique architectures and properties.[2]

Food Waste Valorization

About 250,000 tons of chicken eggshell waste is produced annually worldwide. Eggshell waste is produced at large scale by the processed food industry (*e.g., m*ayonnaise and bakery). Organic waste disposal represents costs and environmental issues. One of the potential solutions to mitigate this problem is to use industrial eggshell waste as a precursor of hydroxyapatite (HAp), one of the main components of the human bones (~60wt%).[3]

Previous Results & New Approach

In order to contribute to this field, Dr. Movil and their students worked previously on the fabrication of HAp from waste eggshells. They incorporated the fabricated ceramic material into PLA composite filaments, which were fed into a FDM 3D printing machine to fabricate different scaffolds structures (Fig. b).

However, due to the brittle behavior of the fabricated composite filaments, it was not possible to 3D print scaffolds with HAp contents higher than 3 wt%. The maximum % of filler was very low considering that the target was ~60 wt% of HAp to mimic the human bone composition.

To overcome these limitations, the present work was focused on a technique known as sacrificial mold technique. In this case, the mold were filled with a polymeric solution containing HAp. After drying, the mold was dissolved in hot water, to obtain the desired scaffold (Figure 1c).

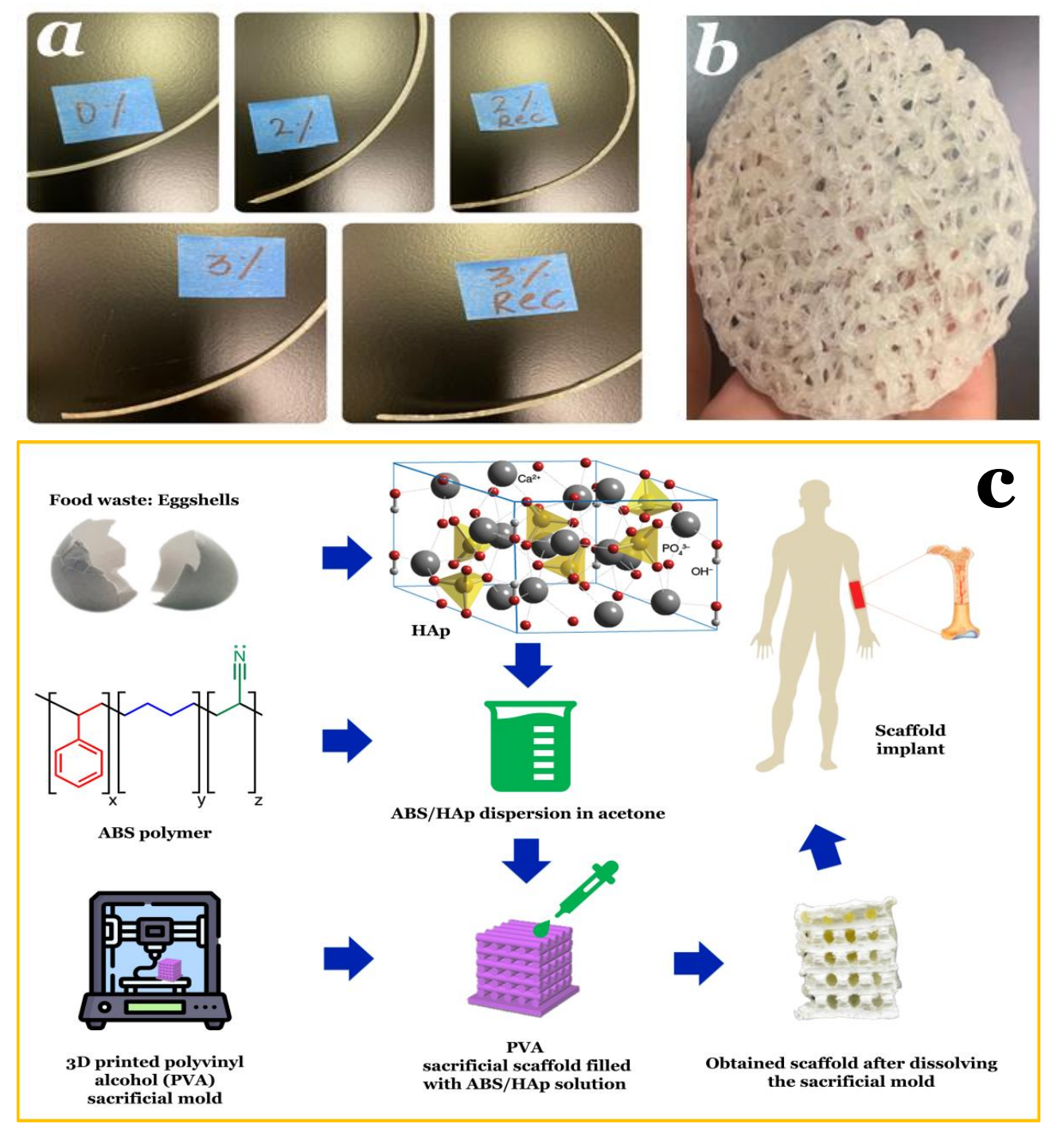

Figure 1. (a) HAp/PLA composite filaments, and (b) HAp/PLA composite scaffold (both fabricated at the PUPR SSMART Lab), and (c) new proposed approach

OBJECTIVES

This research project has three main objectives:

1. Fabricate and characterize HAp from eggshell waste.
2. Establish the process conditions to fabricate ABS/HAp scaffolds with high loads of HAp via 3D printed sacrificial mold method.
3. Study the relationship between composition, microstructure, and mechanical properties of the fabricated scaffolds.

METHODOLOGY

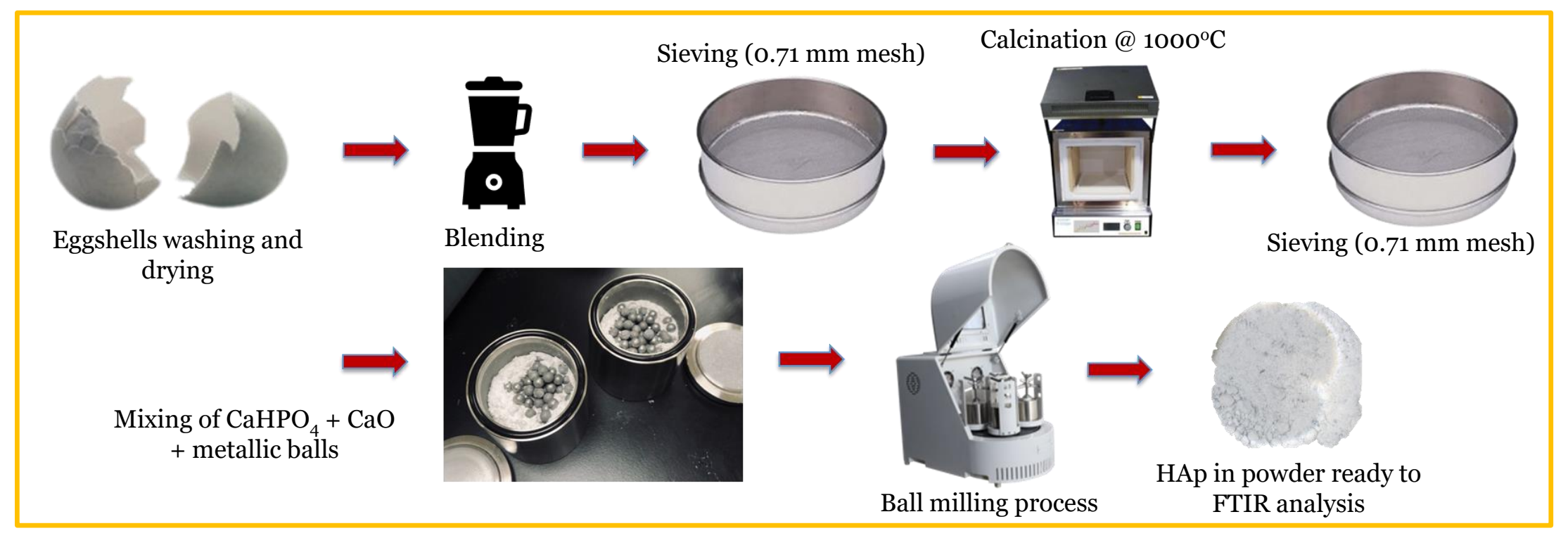

Figure 2. HAp fabrication process

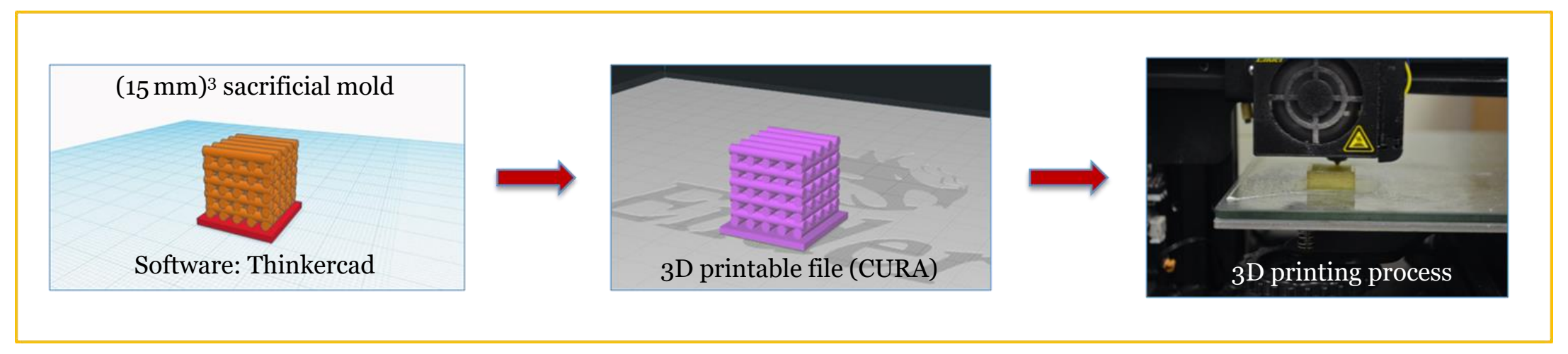

Figure 3. Sacrificial mold fabrication process

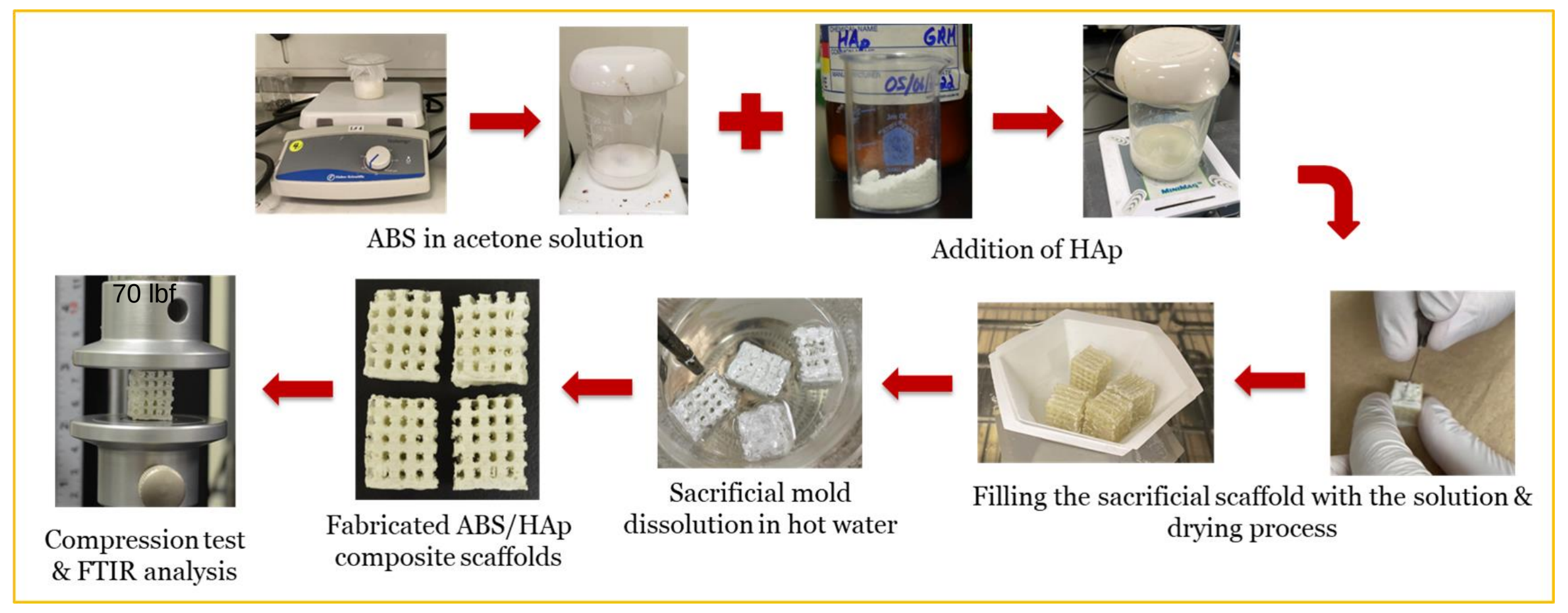

Figure 4. ABS/HAp scaffold fabrication process

Dog-bone-shaped sacrificial molds were also 3D-printed using a commercial PVA filament. The molds were filled with the HAp/ABS solutions and after drying and mold dissolution in hot water, the specimens were obtained. These were fabricated for the tensile tests analysis.

RESULTS

HAp Materials Characterization

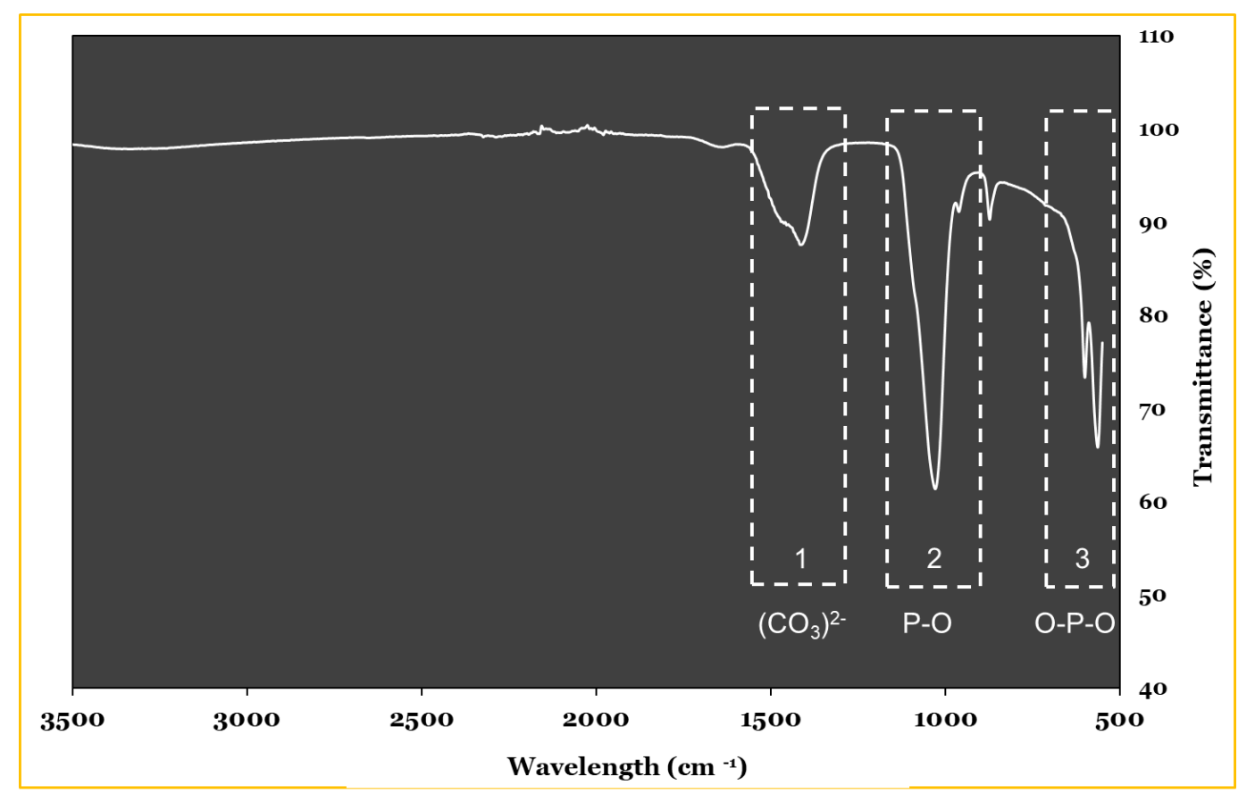

Figure 5. FTIR spectrum of the obtained HAp

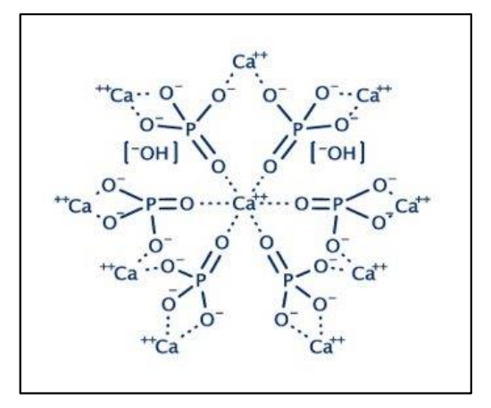

Figure 6. Chemical structure of HAp

Table 1. HAp FTIR spectrum analysis

HAp		
Peak # 1	Wavelength (cm⁻¹)	Description
1	1434 - 1412	Impurity: stretching of carbonate groups $(CO_3)^{2-}$
2	1023 - 965	P – O stretching
3	574 - 560	O – P – O stretching

Fabricated Specimens

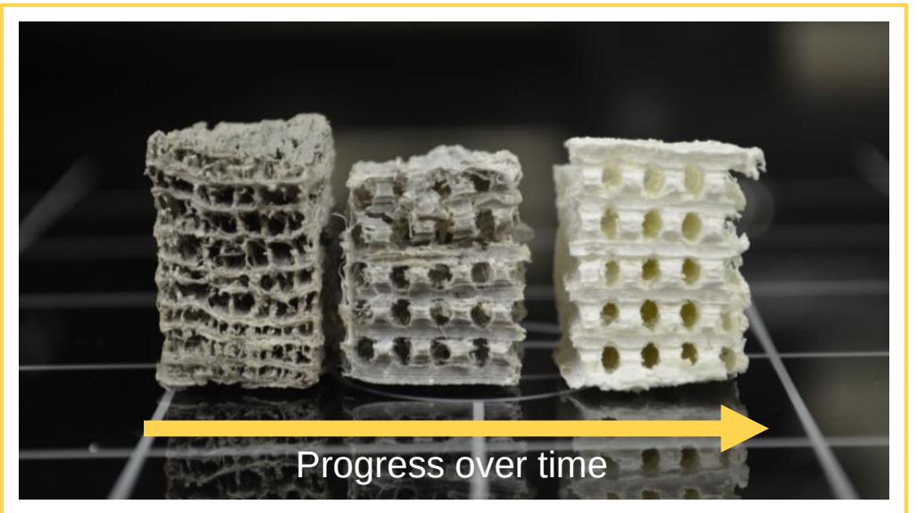

Figure 6. scaffolds fabrication improvements

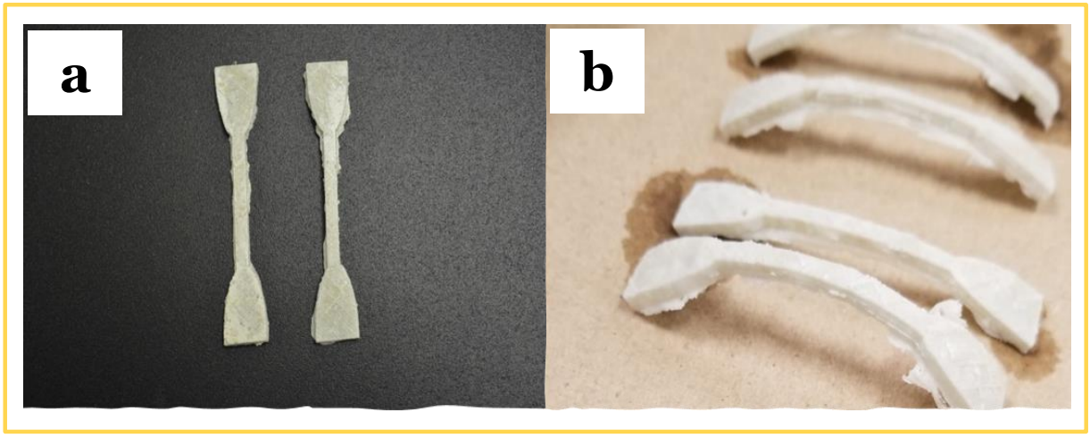

Figure 7. dog-bone-shaped specimens fabricated from (a) ABS, and (b) ABS/HAp solution at 50 wt% HAp

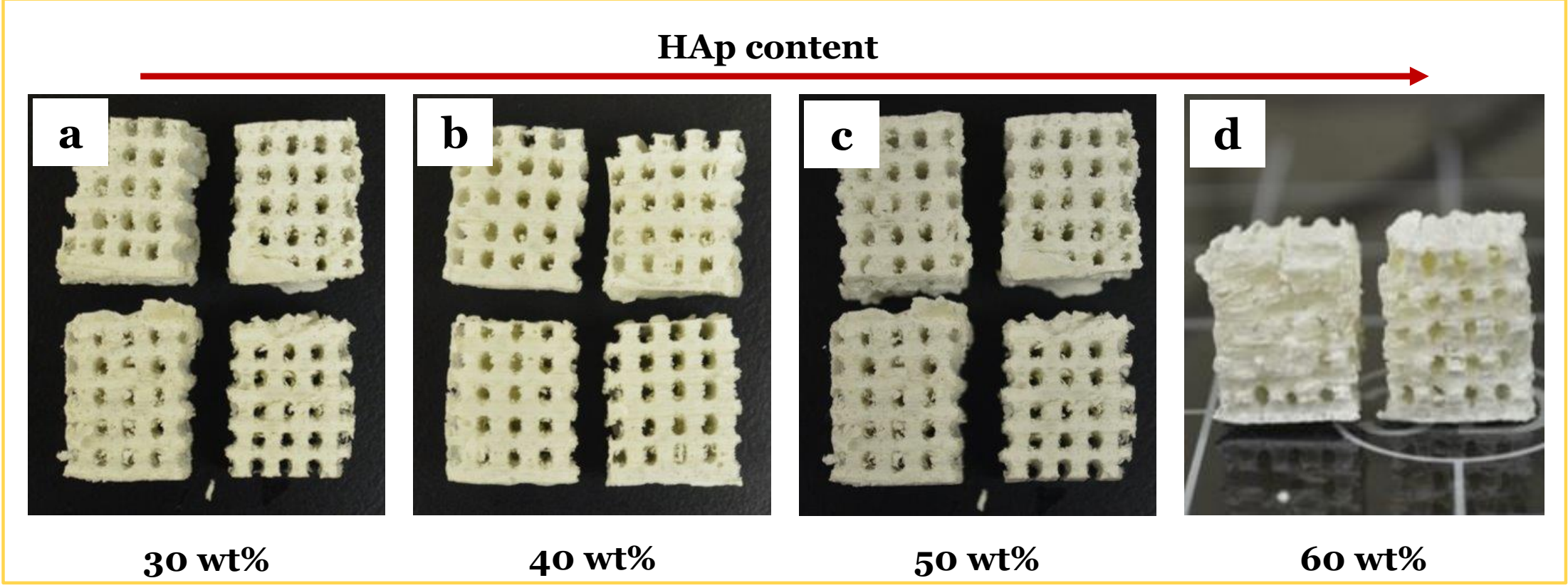

Figure 8. Fabricated scaffolds

RESULTS (Cont.)

Chemical Characterization of the fabricated Composites

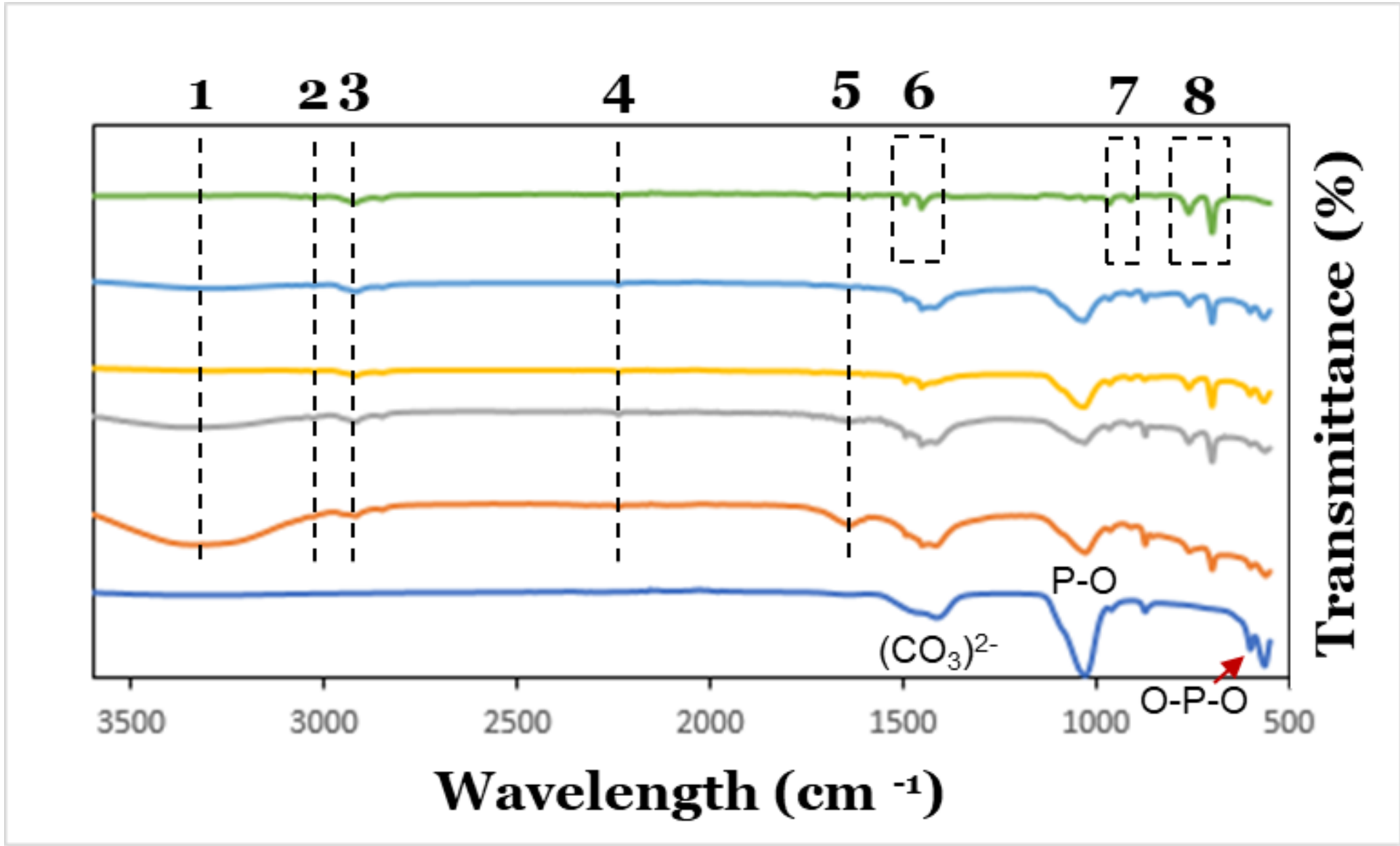

Figure 9. FTIR spectra for the fabricated ABS/HAp composite materials

Table 2. ABS/HAp composites FTIR spectrum analysis

ABS/HAp		
Peak # 1	Wavelength (cm⁻¹)	Description
1	3000 - 3500	Broad peak for O-H bonds (water)
2	3200 - 3000	Aromatic C-H bonds
3	3000 - 2800	Aliphatic C-H bonds
4	2237	C≡N: bonds present in butadiene
5	1638	C=N bonds present in butadiene
6	1650 - 1450	Aromatic ring
7	967 - 911	C-H deformation for H atoms attached to alkene carbons
8	900 - 625	Out-of-plain C-H vibration

Mechanical Characterization of 3D Printed Composite Samples

Since the scaffolds at 60 wt% HAp was so brittle, it was not possible to determine their compressive modulus.

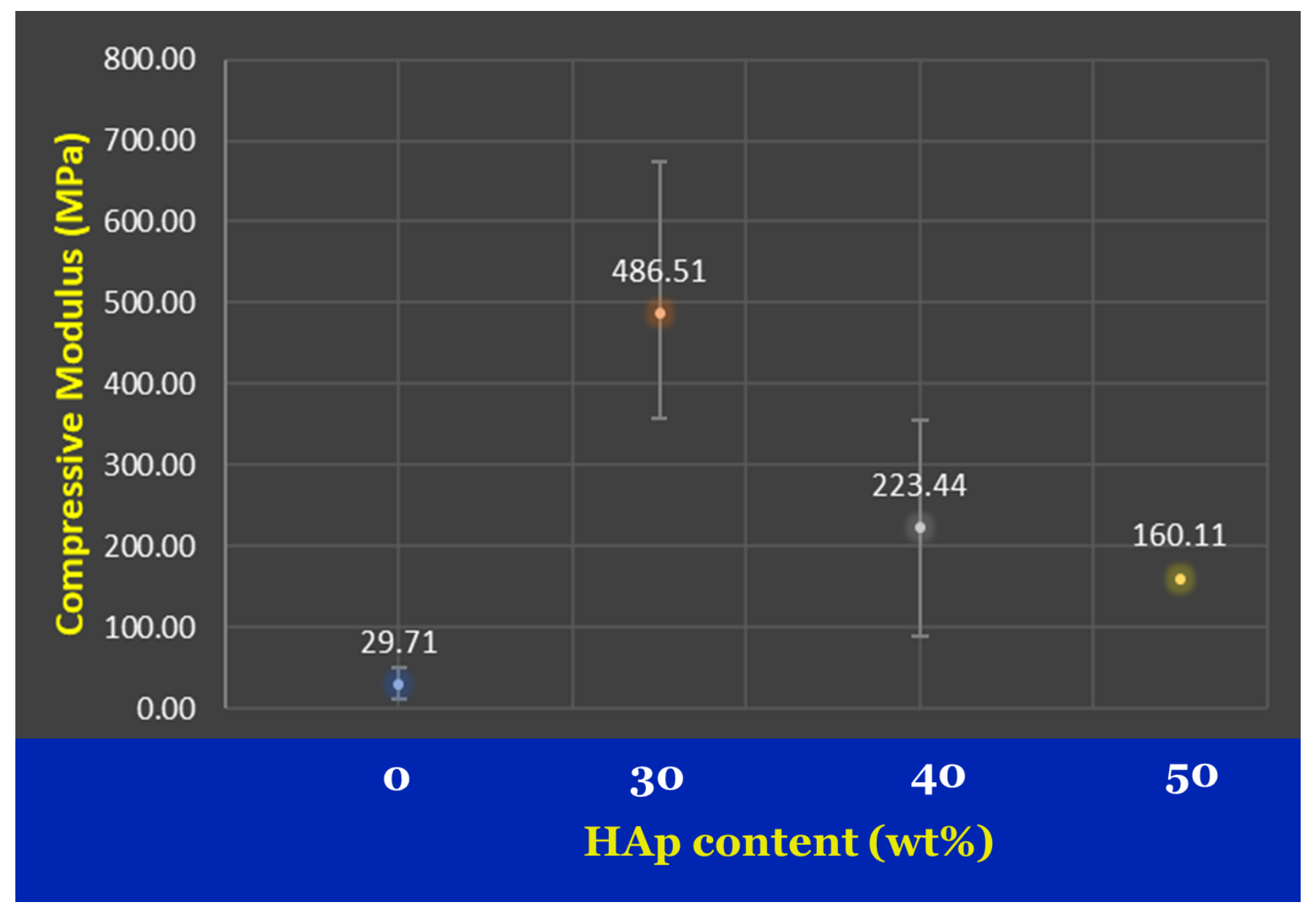

Figure 10. Compressive modulus for the fabricated ABS/HAp scaffolds

Important: The interaction between HAp and the ABS polymer matrix enhances the compression modulus of the composite.

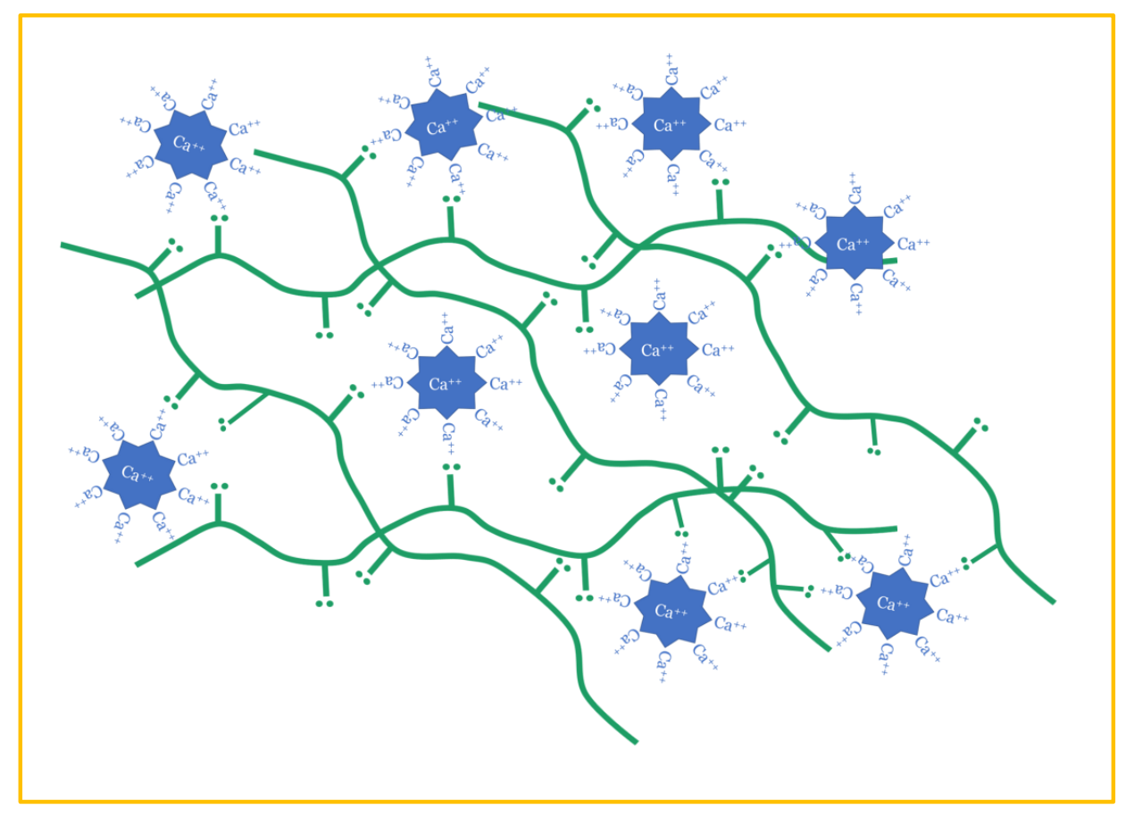

Figure 11. Proposed mechanism for the interaction between HAp (blue) and the ABS polymer (green), which results in the formation of entanglements (cross-linking). ABS (green) and HAp (Blue)

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.
- Perform the compression test at higher forces, since during the experiments the samples did not experience full compression (breaking).
- Study the microstructure of the fabricated specimens via Scanning Electron Microscopy (SEM) to understand their mechanical, electrical, and biological performance.
- Fabricate scaffolds at 60 wt% HAp using the bioprinter recently acquired at the SSMART Lab.

Figure 12. Bioprinter

CONCLUSIONS

- HAp was successfully fabricated from waste eggshell via calcination and subsequently, dry ball milling.
- ABS/HAp scaffolds having compositions of HAp between 0 wt% to 60 wt% were fabricated via a sacrificial mold method. At a composition of 60 wt% of HAp, the scaffolds were so brittle to be examined in the compression tester.
- After integrating HAp fillers into the ABS, the composite materials in solution exhibited higher viscosity (qualitative) as compared to ABS solutions, which made difficult the process of filling the sacrificial molds.
- The fabrication of the ABS/HAp dog-bone-shaped specimens with the required quality was impossible to obtain. After dissolving the sacrificial mold (made of PVA), the release of stress in the specimens caused bending of them.
- Compresion test analysis suggests that the compression modulus of the 3D printed specimens increases with the HAp content up to 30 wt%. At higher HAp contents, the scaffolds exhibited a reduction of their stiffness. It is believed that the excess of cross-linking that took place between the polymer matrix and the HAp particles made the material more brittle.

RECOMMENDATIONS

- It is recommended to perform XRD experiments to corroborate the formation of HAp, determine its crystallinity and detect impurities.
- It is also recommended to perform TGA and DSC analysis to evaluate if the presence of high amounts of HAp has any effect on the thermal behaviour of ABS, specially on its glass transition temperatures.
- Quantify the viscosity of the ABS/HAp solutions at different HAp contents to be able to control the sacrificial mold filling process.
- The use of more sophisticated 3D bioprinters could increase the quality of the specimens.
- It would be valuable to study the effect of the porosity (empty volume) of the scaffolds on their biological and mechanical behaviour.
- 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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