Cellular and Mechanical Performance of 3D-Printed Poly(Lactic acid) and Cocoa Bean Shell-Based Bone Scaffolds

Andres J. Garcia-Brand [1,2*], Alejandro Maranon [3], Carolina Muñoz-Camargo [1], Juan C. Cruz [1] and Alicia Porras [2]

[1] Department of Biomedical Engineering, Universidad de los Andes, Bogota, Colombia. [2] Department of Mechanical Engineering, Universidad de los Andes, Bogota, Colombia. [3] Department of Chemical and Food Engineering, Universidad de los Andes, Bogota, Colombia. ***Corresponding author: aj.garcia14@uniandes.edu.co**

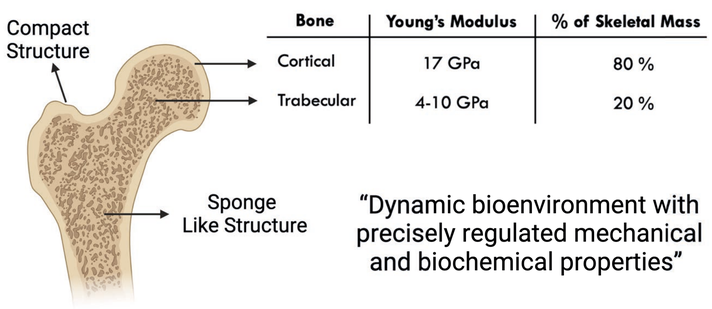
Polymers 2022

ABSTRACT

Bone scaffolds fabricated using additive manufacture has significant advantages over conventional manufacturing methods because they provide routes to form porous structures with customizable architectures that could be potentially exploited as bone fillers. The prevalent biomaterials for the three-dimensional (3D) printing of bone fillers are amorphous polymers such as polylactic acid (PLA) and polycaprolactone (PCL), which are generally filled with calcium phosphates and nanostructured materials as osteoinductive and osteoconductive excipients. However, despite the recently developed cutting-edge alternatives to improve surface bioactivity and bone microenvironment mimicking, there are still issues regarding their limited ability to promote cell attachment and low tensile and compressive strength. A promising avenue to overcome these issues is the use of polysaccharide-rich filler such as natural fibers (NFs), which have demonstrated improving significantly tensile properties and might exhibit antioxidant potential without compromising their biocompatibility, as is the case of cocoa bean shells (CBS). Hence, this work combined the fabrication of a sustainable and low-cost PLA and CBS filled composite with a Fused Deposition Modeling (FDM) approach via filament-based 3D printing to fabricate bioactive cylindrical bone scaffolds. The manufactured composite scaffolds were characterized via tensile and compressive performance tests, together with in vitro Vero and Human Osteoblasts cell attachment and proliferation assays. The results showed tunable the tensile and compressive strength values by adding the CBS, approaching those of trabecular bone. Phalloidin and DAPI cell staining in vitro demonstrated an increase of viable cell attachment and proliferation after 15 days. Compared with previously introduced PLA-based composites for 3D printing of bone fillers, our PLA/CBS composites provide a low-cost, eco-friendly, and bioactive alternative to overcome lack of bioactivity and low mechanical performance. We are certain that the newly developed polymeric bone scaffolds hold much promise for delineating much more personalized treatments for addressing bone failures and defects.

INTRODUCTION

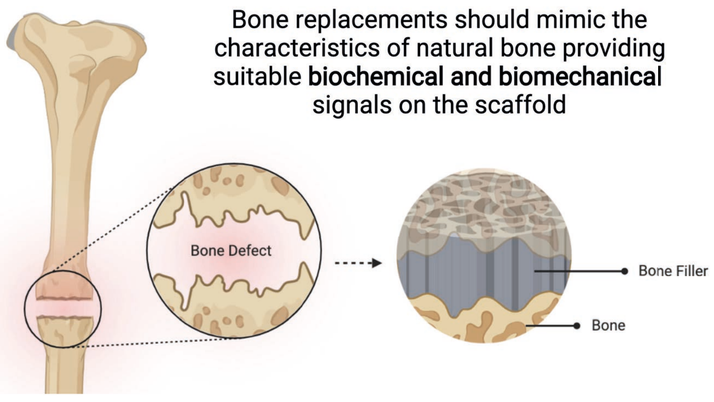
1. Human Bone



Bone	Young's Modulus	% of Skeletal Mass
Cortical	17 GPa	80 %
Trabecular	4-10 GPa	20 %

"Dynamic bioenvironment with precisely regulated mechanical and biochemical properties"

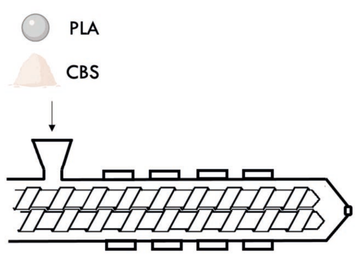
2. Bone Fillers and Replacements



Bone replacements should mimic the characteristics of natural bone providing suitable **biochemical and biomechanical** signals on the scaffold

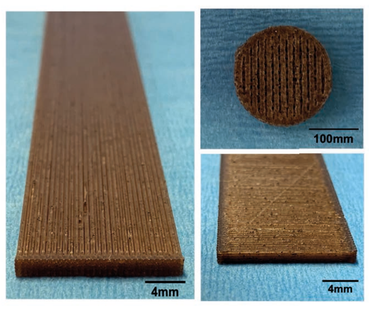
METHODS

1. Extrusion



Twin-Screw Extruder

2. 3D Printing



3. Compressive and Tensile Tests

ASTM D3939 | ASTM D695 – 15

3. Cell Culture

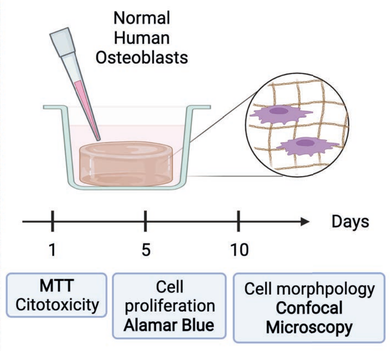
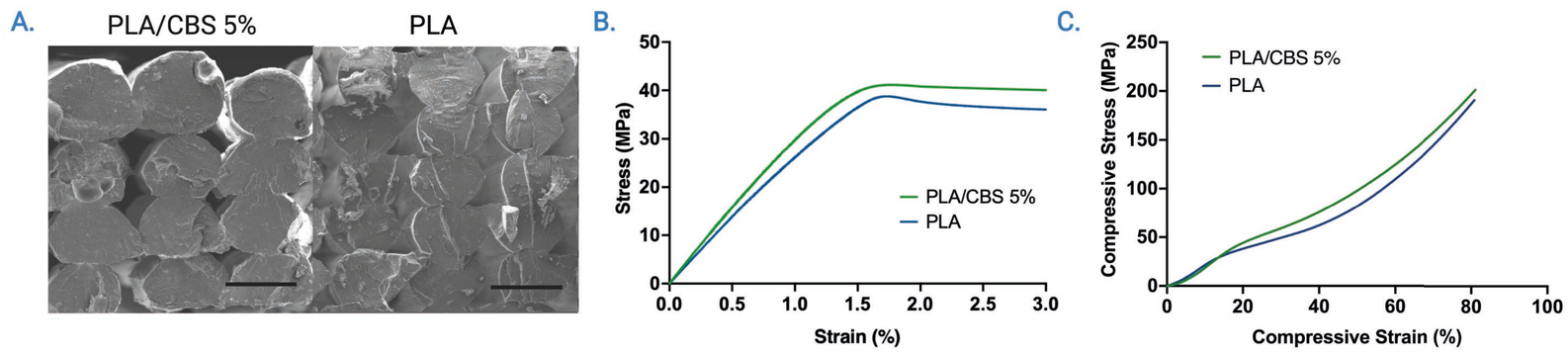


Figure 1. Fabrication and testing of PLA/CBS 5% composites. 1) 3D printing filament manufacture using a twin-screw extruder. 2) FDM of compressive and tensile test probes and bone scaffolds. 3) Mechanical characterization. 4) in vitro cell response validated by Normal Human Osteoblast cell seeding. Citotocicity was evaluated using MTT assay, cell profileration by Alamar Blue assay and cell morphology and proliferationn by fluoresent stainningn and confocal microscopy.

RESULTS



D.

Sample	Tensile Modulus (GPa)	Tensile Strength (MPa)
PLA	2.936 ± 0.172	41.082 ± 2.270
PLA/CBS 5%	2.824 ± 0.325	37.982 ± 2.383
Cortical Bone	13 - 50	52 - 151
Trabecular Bone	4 - 30	10 - 20

E.

Sample	Compressive Modulus (GPa)	Compressive Strength (MPa)	Maximum Compression (%)
PLA	0.030 ± 0.0012	0.121 ± 0.027	6.727 ± 0.532
PLA/CBS 5%	0.034 ± 0.0039	0.142 ± 0.041	7.127 ± 0.724
Cortical Bone	11.5 - 17	130 - 200	NR
Trabecular Bone	0.05 - 0.5	0.1 - 16	NR

Figure 2. Microscopic and mechancial characterization of 3D printed samples. **A.** Scanning Electron Microscopy (SEM) grpahs of cryofractures tensile specimens. Scale bar corresponds to 5 mm **B.** Representative tensile strain-stress curves of 3D printed tensile samples with 100% filling.**C.** Representative compressive strain-stress curves of 3D printed cilindrical samples. **D.** Tensile properties. **E.** Compressive properties. Tensile and compressive properties of travecular and cortical bone are shown to compare the resultant properties with the final application as bone filler.

Microscopic and Mechanical Performance

1. PLA/CBS 5% filaments shows promising results for 3D printing applications since they provide comparable SEM graphs than those of PLA.

2. Tensile and compresive properties of 3D printed specimens are suitable for trabecular bone replacement by their corresponding ultimate strength and Modulus.

Cellular Performance:

1. The incorporation of CBS into the PLA seems to improve ostoblast prolifereation after 7 days possibly due to the extractable compounds of CBS rich in polyphenols and akaloids.

2. CBS provides cellular adhesion sites by the presence of cellulose, lignin and hemicellulose that enhace integrin binding and by so, cell elongation.

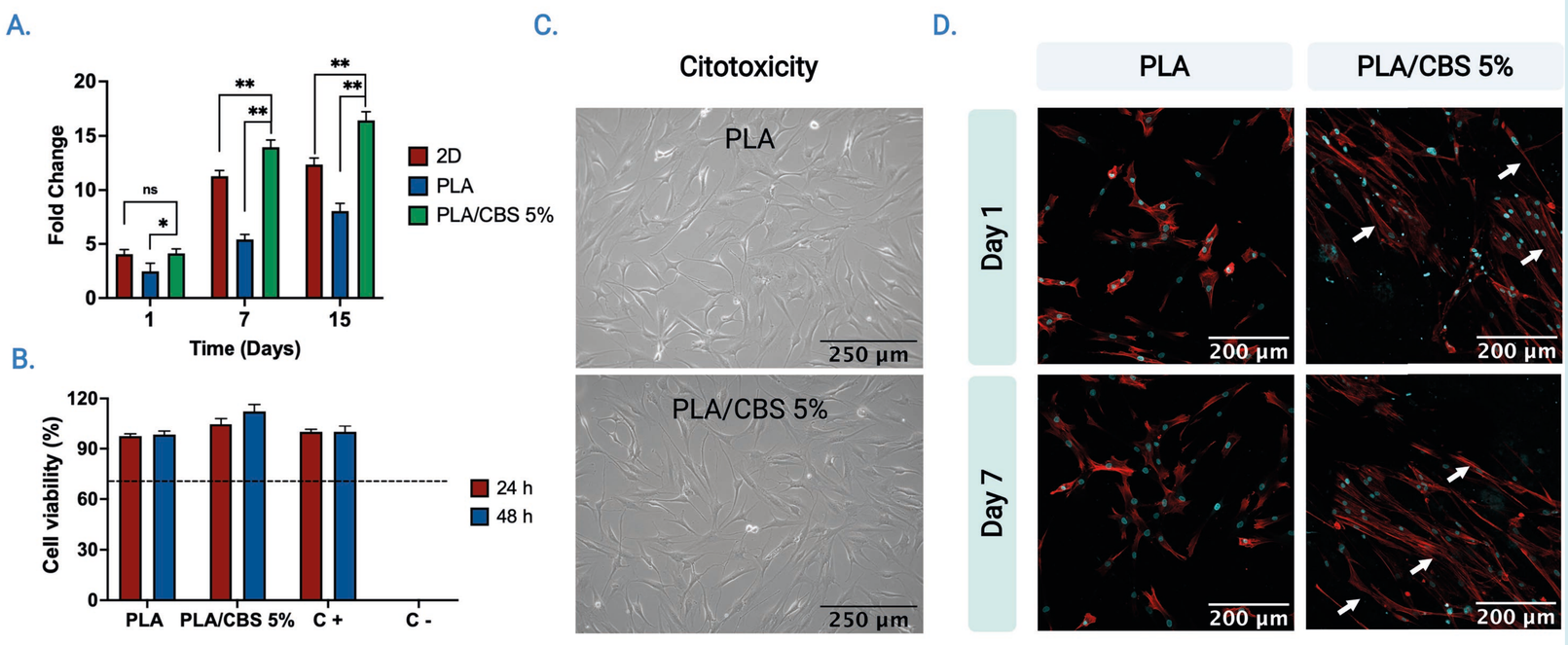


Figure 2. in vitro cellular behaviour of samples with Normal Human Osteoblast (NHOst). **A.** Cell proliferation capacity detected by AlamarBlue assay. 2D cell culture was used as control. **B.** Cell viability after 24 and 48 h of direct contact with square samples quantifyied by MTT citotoxicity assay. DMSO 10% and Osteoblast Basal Medium (OBM) was used as positeve and negative controls of citotoxicity, respectively. **C.** Cell morphology after direct contact with square samples. **D.** Confocal images of infiltrated NHOst in the 3D printed scaffolds. Red: F-actin fillaments, Blue: Cell-nuclei. White arrows show cells with the correct elongated morphology (strongly adhered to the matrix.

CONCLUSION

The PLA/CBS composites containing 5 %wt. showed to be advantageous for regeneration as the scaffold showed promising results to be used as bone fillers, as confirmed by Alamar blue, and MTT citotoxicity assays and DAPI/ Phalloidin. Moreover, the addition of CBS into the PLA matrix seems to increase both tensile and compressive Modulus. A beneficial property to tune the scaffold stiffness to approach the mechanical properties of the native human trabecular bone and thus improve the cellular mechano-trasduction.

REFERENCES

He, H.; Cheng, M.; Liang, Y.; Zhu, H.; Sun, Y.; Dong, D.; Wang, S. Intelligent Cellulose Nanofibers with Excellent Biocompatibility Enable Sustained Antibacterial and Drug Release via a PH-Responsive Mechanism. J. Agric. Food Chem. 2020, 68, 3518–3527, doi:10.1021/acs.jafc.9b06588.

Porras, A.; Maranon, A.; Ashcroft, I.A. Characterization of a Novel Natural Cellulose Fabric from Manicaria Saccifera Palm as Possible Reinforcement of Composite Materials. Compos. Part B Eng. 2015, 74, 66–73, doi:10.1016/j.compositesb.2014.12.033.

Wollgast, J.; Anklam, E. Review on Polyphenols in Theobroma Cacao: Changes in Composition during the Manufacture of Chocolate and Methodology for Identification and Quantification. Food Res. Int. 2000, 33, 423–447, doi:10.1016/S0963-9969(00)00068-5.