

# Assessment of *in vitro* degradation of 3D

# bioreabsorbable scaffolds for bone regeneration

Evelina Pacheco<sup>1</sup>, Marcela Arango-Ospina<sup>2</sup>, Aldo R. Boccaccini<sup>2</sup>, Élica B. Hermida<sup>1</sup> and Beatriz Aráoz<sup>1</sup>

<sup>1</sup>Institute of Emerging Technologies and Applied Sciences (ITECA), National Scientific and Technical Research Council (CONICET), National University of General San Martín (UNSAM), San Martín 1650, Buenos Aires, Argentina

<sup>2</sup>Department of Materials Science and Engineering, Institute of Biomaterials, University of Erlangen-Nuremberg, 91058 Erlangen, Germany

## INTRODUCTION & AIM

The human body has an innate ability to heal itself in the case of minor skin wounds or bone fractures. However, the treatment for extensive injuries requires the development of three-dimensional structures, known as scaffolds, that allow cells to adhere, proliferate, differentiate, and regenerate the extracellular matrix. Polymers can be combined with bioactive agents to create 3D printed scaffolds that mimic natural bone architecture and patient anatomy. In this context, the combination of polyhydroxybutyrate-co-hydroxyvalerate (PHBV) with bioactive glasses (BG1393 and BG45S5) presents a promising strategy for bone tissue engineering.

### PHBV:

- Biocompatible, non-cytotoxic and biodegradable (~ 2 years)
- Stiffness (0.2 - 1.5 GPa)

### Bioactive glasses:

- Osteoconductive and osteoinductive
- Enables hydroxyapatite formation

The aim of this study is to evaluate the influence of filler content on morphology and mechanical properties during *in vitro* degradation.

## METHODS

PHBV + BG (0, 2 and 4 wt%, 1393 and 45S5 compositions)

Filaments extruded in ThermoScientific Process 11 and scaffolds 3D printed by FDM in Codex 2022

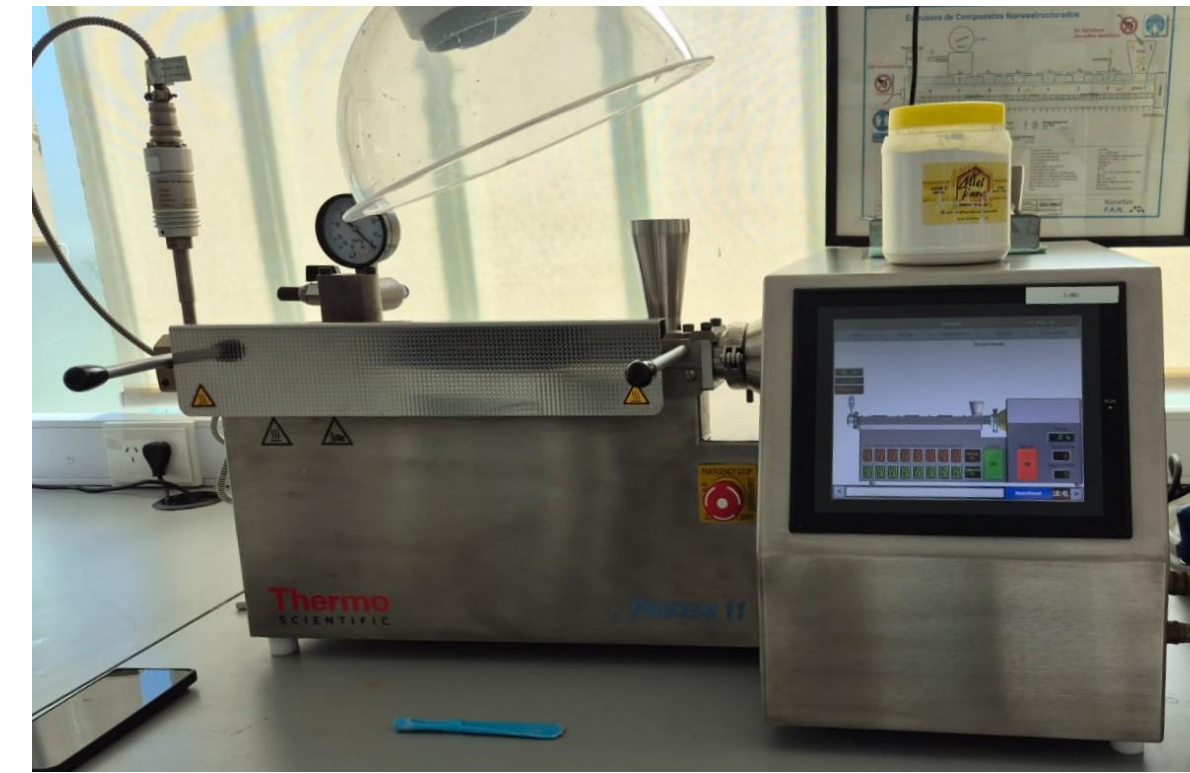


Fig. 1: parallel twin-screw mini-extruder (Thermo Fisher Scientific Process 11)

- Nozzle: 0,5 mm
- Printing speed: 6 mm/min
- Rectilinear pattern
- 55% infill
- Temperatures: bed 90°C and nozzle 195°C

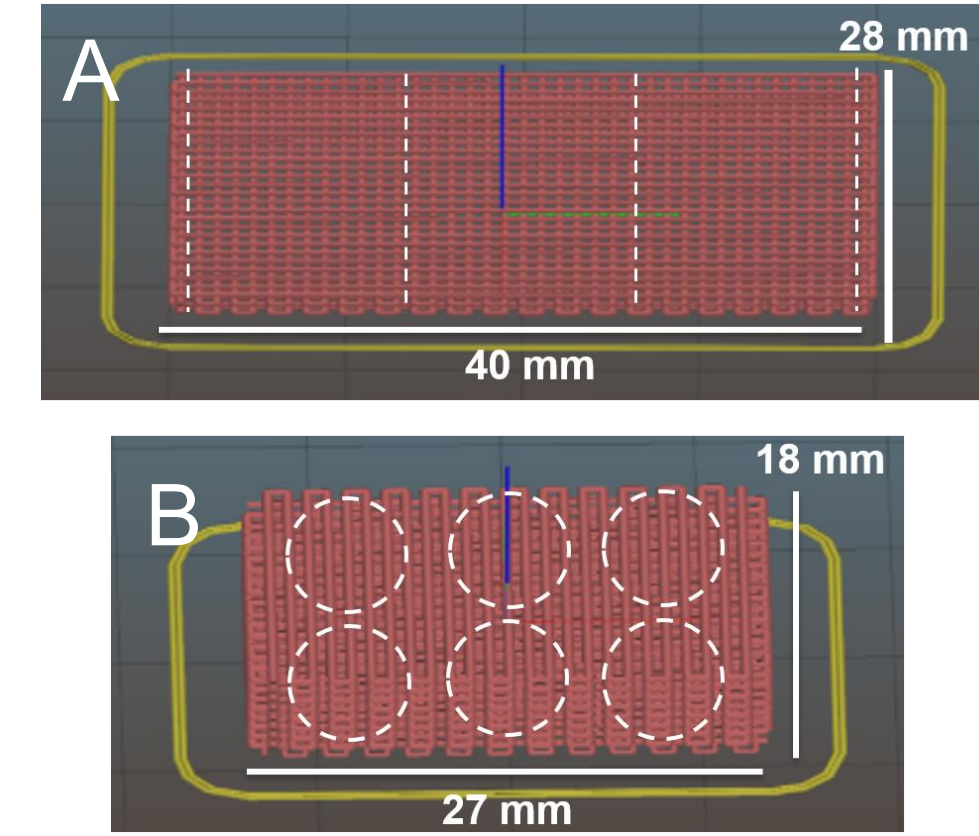


Fig. 2: geometries for A. Bending test (5 layers) and B. Compression test (17 layers)

For *in vitro* degradation, scaffolds were soaked in simulated body fluid (SBF) at 37°C for 30 days. Scaffolds were characterized by optical images, Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDS), weight loss measurements and mechanical behavior under bending (DMA, force ramp rate: 0.5 N/min) and compression (Universal Testing Machine, speed: 1 mm/min).

## RESULTS & DISCUSSION

### FILAMENTS EXTRUSION:

- ✓ Homogeneous and continuous PHBV and PHBV + BG filaments
- ✓ 1.7 ± 0.1 mm in diameter
- ✓ Suitable for Fused Deposition Modeling (FDM) printing

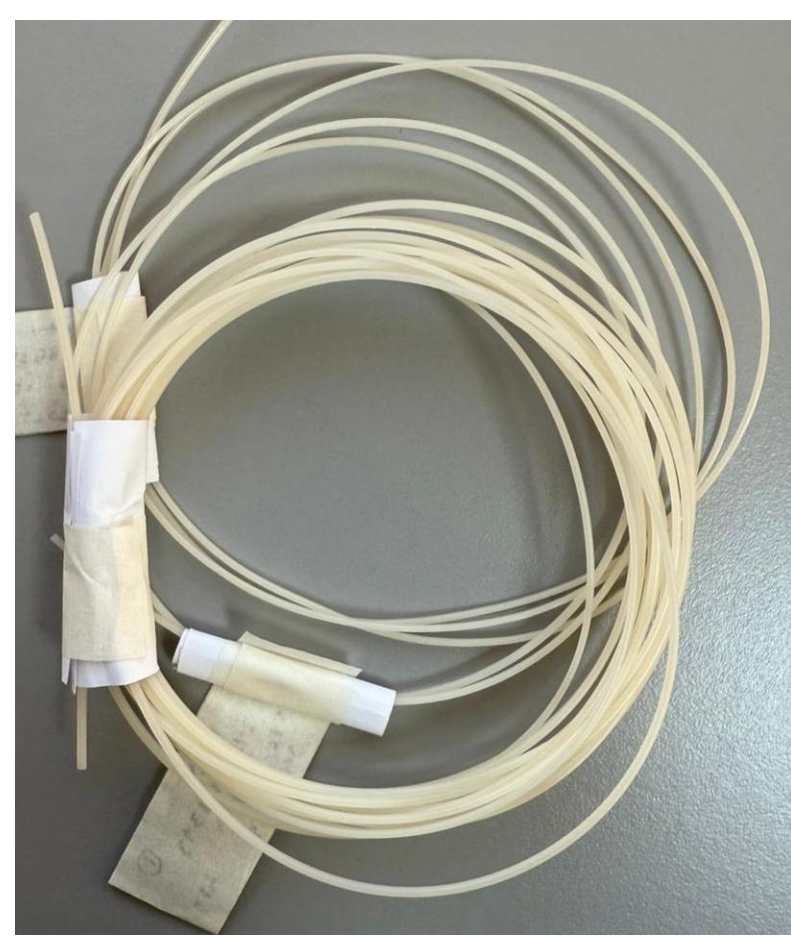


Fig. 3: PHBV and PHBV + BG filaments

- ✓ The printed scaffolds showed high printing quality and good adhesion between layers
- ✓ Interconnected pores with pore size that promotes osteogenesis (100–500 µm)

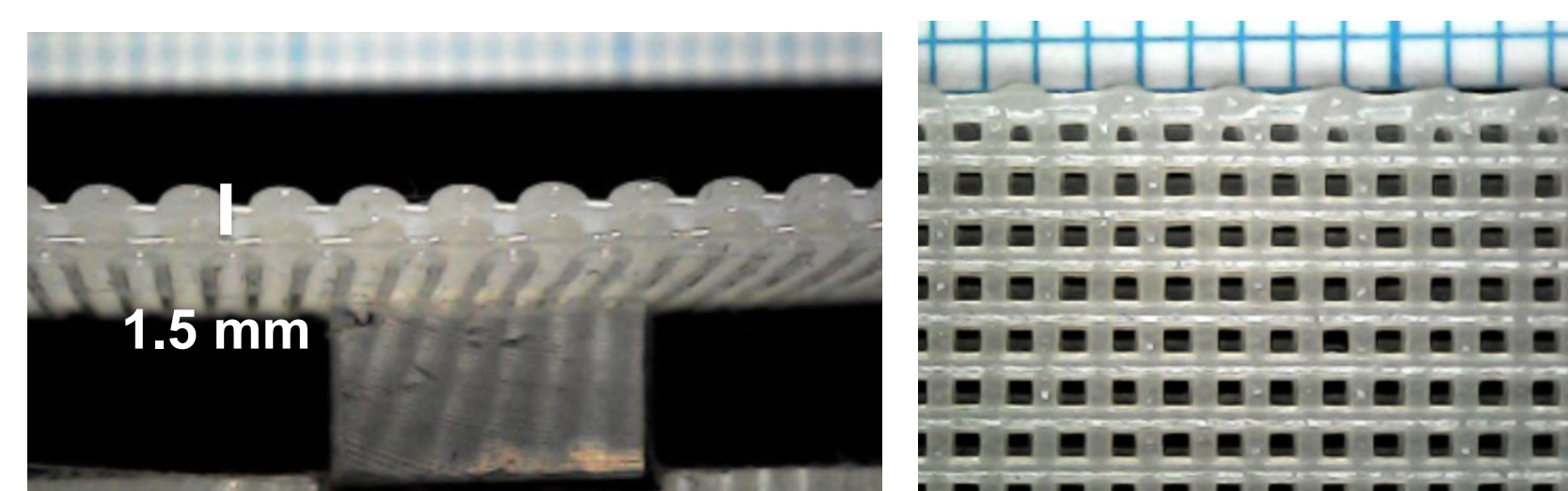


Fig. 4: PHBV and PHBV + BG scaffolds printed for 3 point-bending test (5 layers)

### 3D-PRINTED SCAFFOLDS:

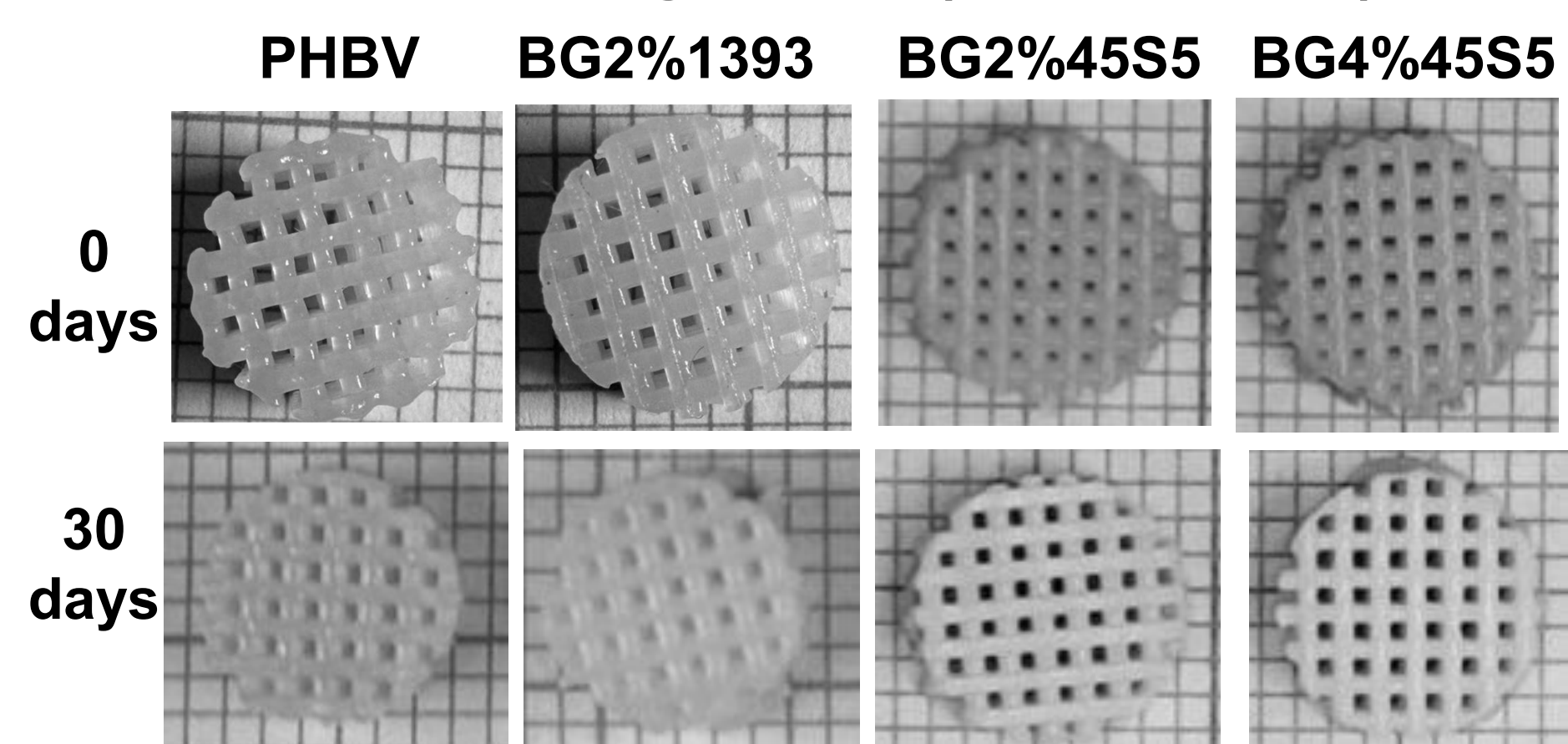


Fig. 5: PHBV and PHBV + BG scaffolds printed for compression test (17 layers) (0 and 30 days of degradation)

- ✓ During degradation, scaffolds maintained structural integrity
- ✓ Scaffolds with BG 2% and 4% exhibit whitening along degradation, from day 3 of incubation

### BIOACTIVE POTENTIAL:

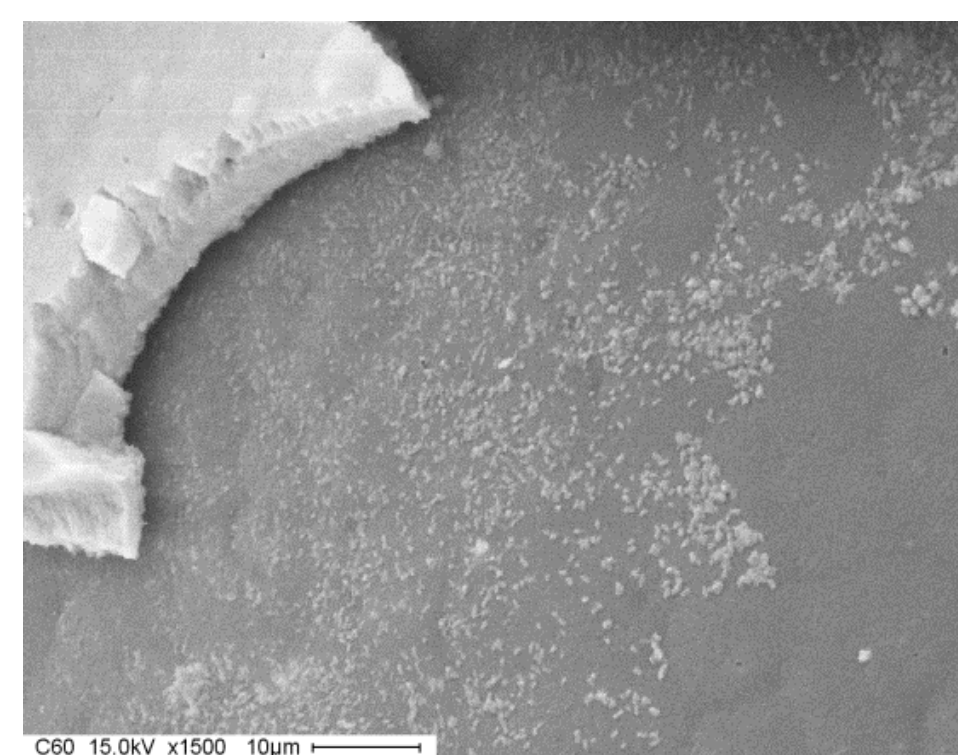


Fig. 6: SEM image of HA formed on PHBV+BG scaffolds after 3 weeks of immersion in SBF

- ✓ BG particles enhanced bioactivity of the composite, leading to the formation of a hydroxyapatite-like layer on the surface (EDS: Ca/P At%=1.60)

- ✓ The elastic modulus in compression and flexure were comparable to those of trabecular bone (0.1–2 GPa)
- ✓ Scaffolds with BGs exhibited signs of loss in their structural integrity under compression (for BG4%45S5 at 3 days of incubation and for BG2%45S5 and BG2%1393 at 15 days of incubation)

Table 2: Elastic moduli of PHBV and PHBV + BG scaffolds

Elastic modulus (MPa)	PHBV	PHBV + 2%BG 1393	PHBV + 2%BG 45S5	PHBV + 4%BG 45S5
In compression	270±70	240±50	300±60	230±60
In compression, after 30 days in SBF	310±60	270±90	410±80	340±50
In bending	380±40	440±40		
In bending, after 30 days in SBF	430±30	460±30		

### MECHANICAL BEHAVIOUR

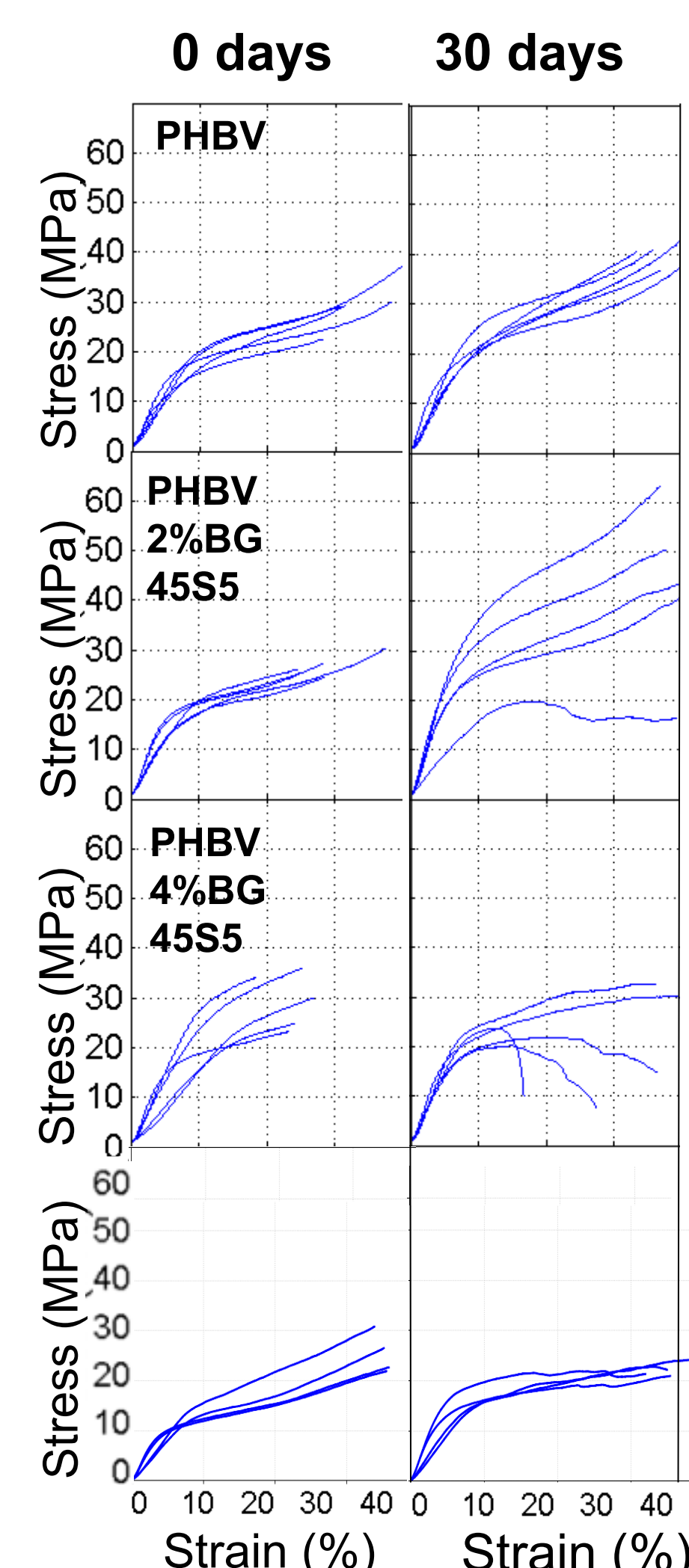


Fig. 7: Compression stress-strain curves of PHBV + BG scaffolds (0 and 30 days of degradation)

### WEIGHT LOSS:

- ✓ Weight loss under 2%

Table 1: Weight loss measurements of PHBV and PHBV + BG scaffolds after immersion in SBF for 30 of PHBV + BG

SBF x 30 days	PHBV	PHBV + 2%BG 1393	PHBV + 2%BG 45S5	PHBV + 4%BG 45S5
Degradation (%)	1.6±0,4	1.2±0,2	1.4±0,4	2.1±0,5

## CONCLUSIONS

This study demonstrates that bioactive glass particles influence the degradation kinetics of PHBV/BG composites, with potential applications in bone regeneration. During *in vitro* degradation, scaffolds maintain their elastic modulus despite signs of fracture under compression, while successfully forming a hydroxyapatite-like layer that enhances bioactivity.

## ACKNOWLEDGMENT

The authors acknowledge the funding of CONICET (Argentina), the German Research Foundation (DFG) (Project BO 1191/41-1), and the Argentine Nanotechnology Foundation (FAN) for the use of the extruder. E.P. thanks CONICET for the PhD fellowship.