

## Supporting Information for

### Oil-in-eutectic mixture HIPEs co-stabilized with surfactant and nanohydroxyapatite: ring-opening polymerization for nanocomposite scaffold synthesis

José M. Cervantes-Díaz,<sup>a</sup> Sergio A. García-Landeros,<sup>a</sup> A. Gutierrez-Becerra,<sup>a</sup> J. Benito Pelayo-Vazquez,<sup>a</sup> Gabriel Landázuri-Gómez,<sup>b</sup> Jorge Herrera-Ordóñez,<sup>c</sup> J. Félix Armando Soltero-Martínez,  
<sup>b</sup> Josué D. Mota-Morales <sup>\*c</sup> and María G. Pérez-García <sup>\*a</sup>

<sup>a</sup> Centro Universitario de Tonalá, Universidad de Guadalajara, Tonalá, Jalisco 45425, México

<sup>b</sup> Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara, Guadalajara, Jalisco 44430, México

<sup>c</sup> Centro de Física Aplicada y Tecnología Avanzada, Universidad Nacional Autónoma de México, Querétaro 76230, México

† Both authors contributed equally

\*Corresponding Authors. Email:

[mariag.perezg@academicos.udg.mx](mailto:mariag.perezg@academicos.udg.mx)

[mota\\_josue@hotmail.com](mailto:mota_josue@hotmail.com)

## **Experimental Section**

### **Materials**

(3S)-cis-3,6-Dimethyl-1,4-dioxane-2,5-dione (L-Lactide, LLA, 98%),  $\xi$ -Caprolactone (CL, 97%), pluronic surfactant F127 (97%), hydroxyapatite nanoparticles (NHA, 97%, average particle size <200 nm), tetradecane (> 99%), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 98%), benzyl alcohol (BnOH, 99%) and methanesulfonic acid (MSA, 99.5%) were purchased from Sigma-Aldrich. All materials were used without further purification.

### **Preparation and characterization of oil- in-eutectic mixture HIPEs**

Eutectic mixture (EM) was prepared by mixing LLA and CL in a 30:70 molar ratio at 90°C until a transparent and homogeneous liquid was obtained. The continuous phase of the HIPEs was prepared by dissolving the surfactant F127 in the eutectic mixture. Then, NHA was added to the EM/ surfactant mixture and vortexed at 3200 rpm for at least 20 min to ensure homogeneous mixing. The amount of surfactant F127 and NHA used were 5, 10 and 20 wt % and 0, 0.5, 1, 1.5, 3, 5 and 7 wt % with respect to the total amount of EM , respectively. Tetradecane accounting for 80 vol% was used as internal phase. HIPEs were prepared by mixing both phases in a 5ml glass vial and vortexing at 3200 rpm at least 10 min at 25°C until homogeneous emulsion was obtained. HIPEs were observed using optical microscopy (Olympus BX51) with a camera QICAM (FAST1394) and the software Linksys 32. The average droplet size was calculated by ImageJ analysis software as the average of 100 image readings.

### **Rheology measurements**

All rheological measurements were performed in triplicates on a controlled stress rheometer (ARG2, TA Instruments) using a cone and plate geometry (1° angle and 60 mm diameter). Stress-sweep and strain-sweep data were collected in triplicates at a frequency of 0.1 Hz at 25°C. A solvent trap cover was used to avoid solvent evaporation during the measurements. All experiments were reproducible within a 2 percent standard deviation.

The zero-shear elastic modulus ( $G'_0$ ) was estimated as the average  $G'$  value at the lowest stress or strain amplitude where  $G'$  approximately plateaus. Lag phase vs shear stress or strain plots were used to determine the yield point where a pronounced increase in the lag phase angle was taken to be the yield point.

### **Nanocomposite macroporous materials synthesis and characterization**

The nanocomposite porous materials were labeled as PHIPE-Y-Z, where Y and Z were the amount of surfactant (5, 10 or 20 wt%) and NHA (0, 0.5, 1, 1.5, 3, 5 and 7 wt%) used to prepare the precursor HIPE, respectively. PolyHIPEs were synthesized by polymerizing the continuous phase (LLA-CL-EM) adding a mixture of DBU/BnOH in a 1:1 molar ratio (5 wt% with respect to the total amount of LLA-CL-EM) to the prepared HIPE. The medium was immediately homogenized by vortexing for about 1 min, and then, a small quantity of MSA (3 wt % with respect to the total amount of LLA-CL-EM) was added. The HIPEs were homogenized by vortexing for an additional 2 min. Ring-opening polymerization was carried out for 6 h at 37°C. After polymerization, the PolyHIPEs were extracted from their vials. The produced monoliths were washed in ambient-temperature hexane, 40 times their original

volume, for 12 h to remove the oil phase and later in ethanol for at least 3 days to remove the surfactant. Finally, the monoliths were dried at room temperature until constant weight was reached.

Conversion was determined by dividing the mass of the dried monolith by its expected mass. Ring opening polymerization was studied by  $^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy using a Bruker DRX-500 spectrometer. Samples (typically 20mg) were dissolved in deuterium chloroform (1ml) and placed in NMR tubes, and the spectra were recorded at room temperature. PolyHIPEs were also characterized by X-ray diffraction (XRD) using a Empyrean diffractometer (CuK $\alpha$  radiation, 0.02° step size and 30 s counting time) and by Attenuated Total Reflectance (ATR) Fourier Transform Infrared spectroscopy directly on an ATR device adapted to a Bruker Alpha FTIR instrument.

The macroporous structure of PolyHIPEs was observed by field emission scanning electron microscopy (FESEM, Mira from TESCAN) at an accelerating voltage of 10 kV. All samples were gold coated. The diameters of the pores were calculated by ImageJ analysis software as the average of 100 image readings. In addition, HIPEs were analyzed by Energy Dispersive X-ray Spectroscopy (EDS) using a Bruker instrument.

Compression tests on the monoliths were carried out according to the standard ASTM D 1621 method in an Instron 4411 with a 5 kN load cell and a platen speed of 1 in min $^{-1}$ . The samples were compressed to 75% of their initial height. Elastic modulus was determined from the initial linear slope of the stress–strain curve. In addition, the stress at yield was recorded to give the crush or compression strength.

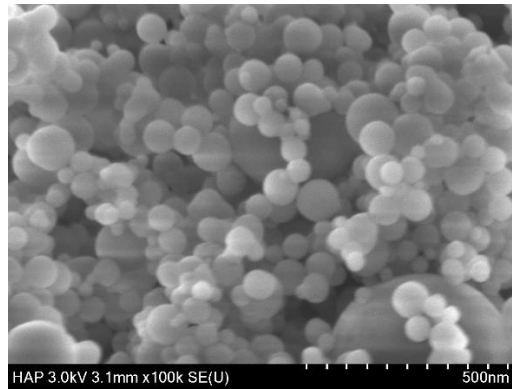


Figure S1. FSEM micrograph of NHA used in HIPEs formulation.

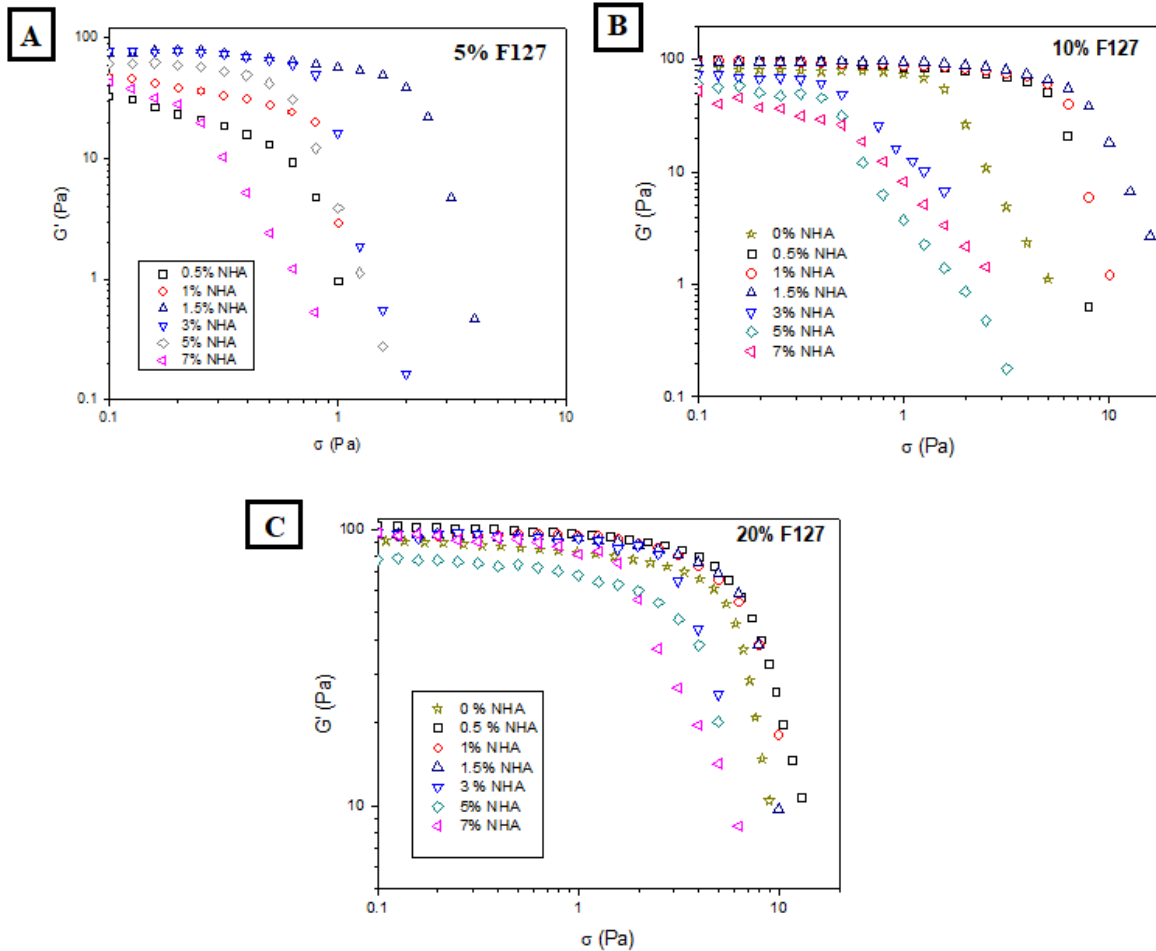


Figure S2. Stress –sweep plots of storage modulus ( $G'$ ) versus shear stress ( $\sigma$ ) for different amounts of surfactant A)5% wt, B) 10% wt and C) 20% wt.

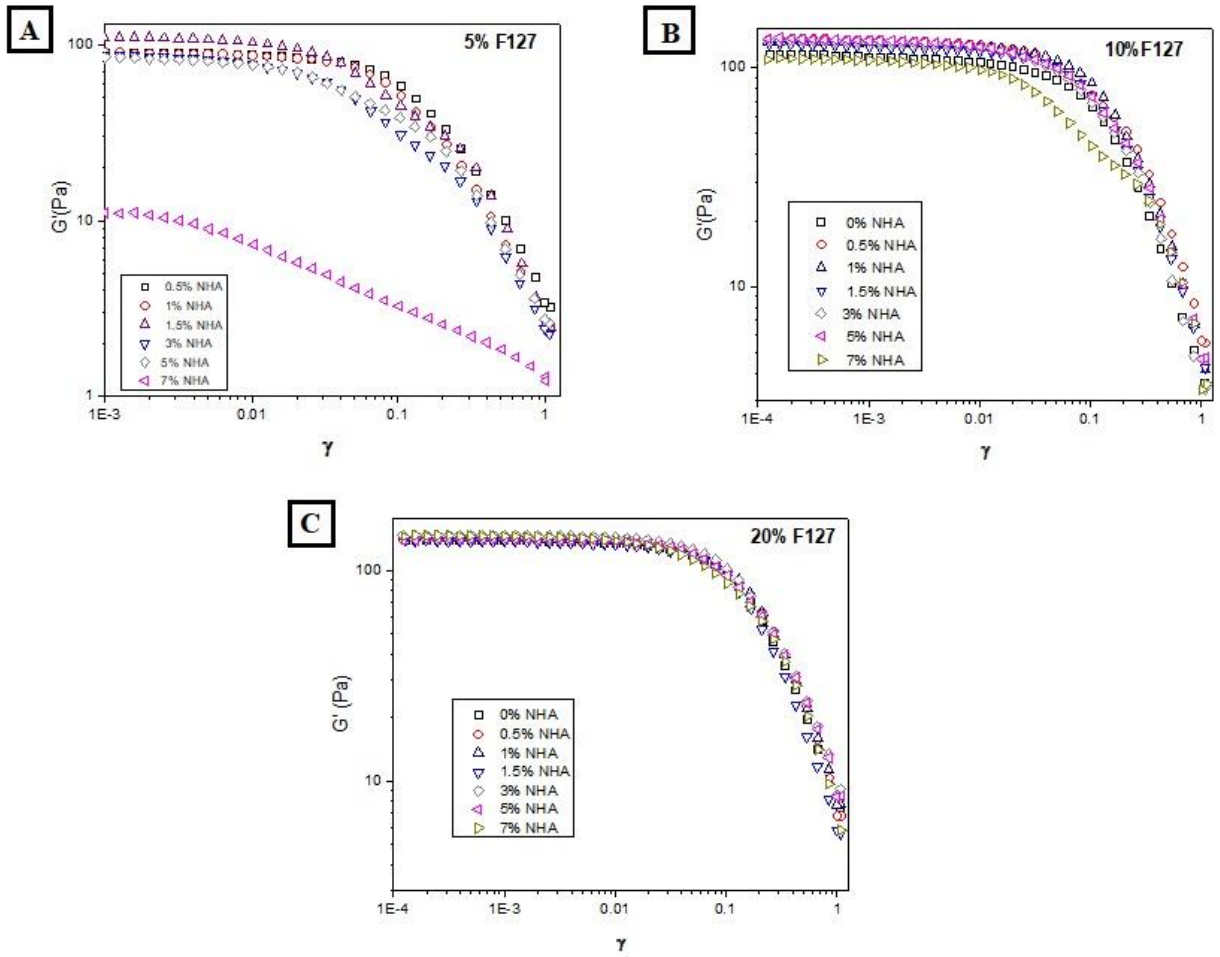


Figure S3. Strain-sweep plots of storage modulus ( $G'$ ) versus strain ( $\gamma$ ) for different amounts of surfactant A) 5% wt, B) 10% wt and C) 20% wt.

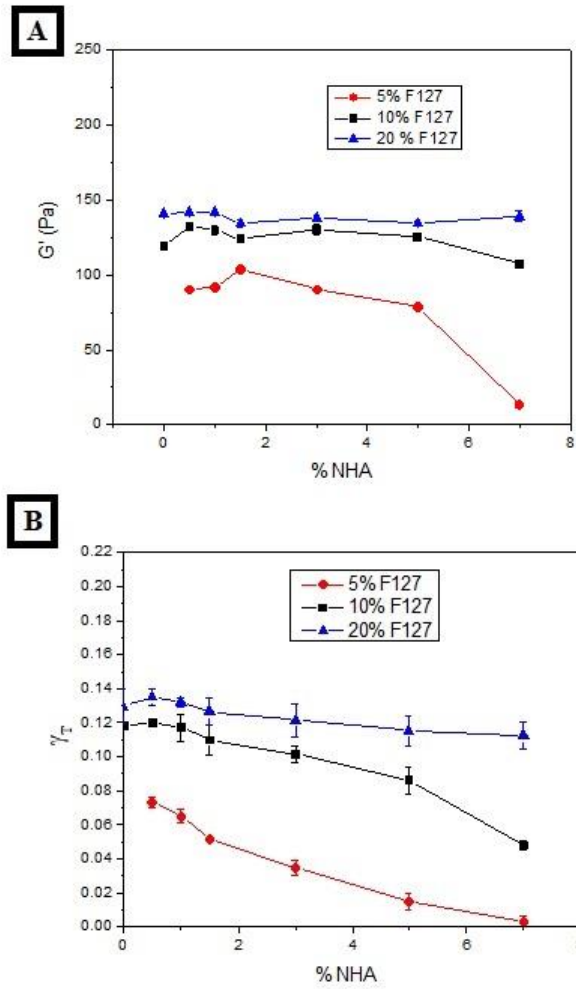


Figure S4. A) HIPE zero-shear elastic modulus ( $G'$ ) and B) HIPE yield strain ( $\gamma_T$ ) as a function of NHA composition for different amounts of surfactant.

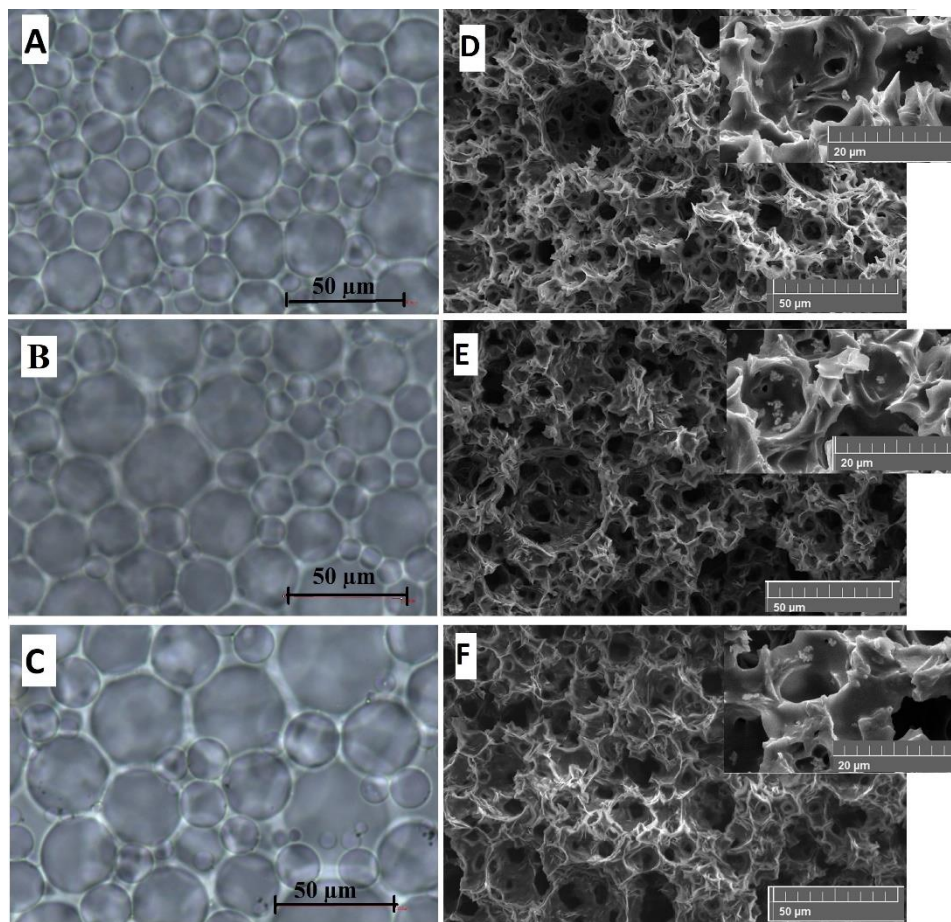


Figure S5. (A, B, C) Optical micrographs of HIPEs: (A) HIPE-5-1, (B) HIPE-5-1.5 and (C) HIPE-5-3. (D, E, F) FSEM micrographs of PolyHIPEs after purification: (D) PHIPE-5-1, (E) PHIPE-5-1.5 and (F) PHIPE-5-3.



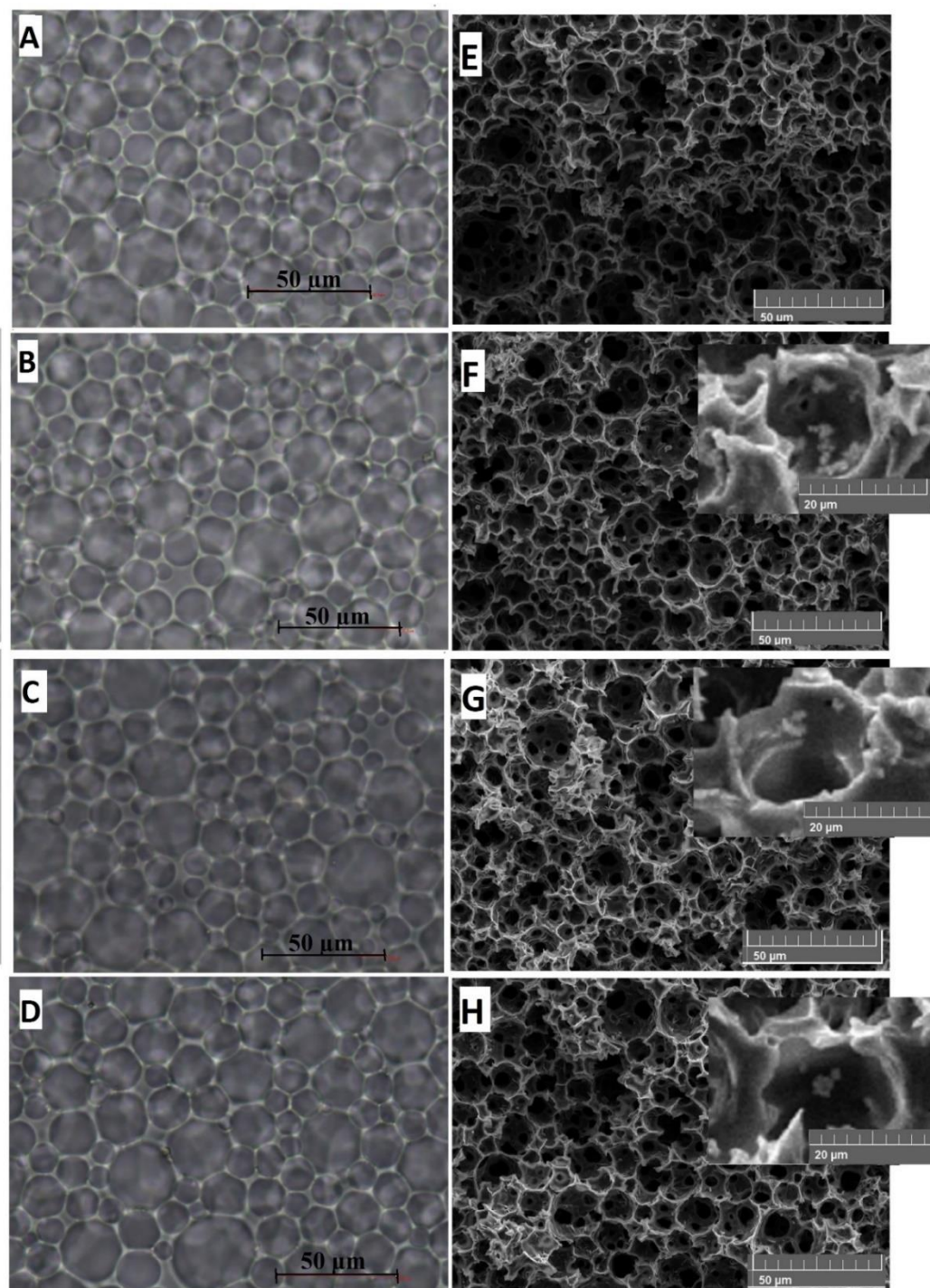


Figure S6. (A, B, C, D) Optical micrographs of HIPEs: (A) HIPE-10-0, (B) HIPE-10-0.5, (C) HIPE-10-1.5 and (D) HIPE-10-3. (E, F, G, H) FSEM micrographs of PolyHIPEs after purification: (E) PHIPE-10-0, (F) PHIPE-10-0.5, (G) PHIPE-10-1 and (H) PHIPE-10-1.5.

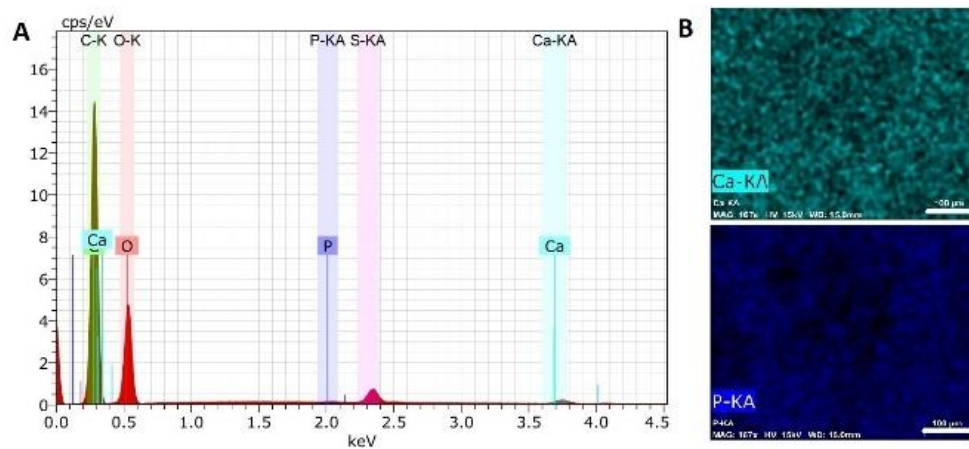


Figure S7. A) EDS spectra and B) elemental mapping of PHIPE-10-1.5 (scale bars are 100 μm).

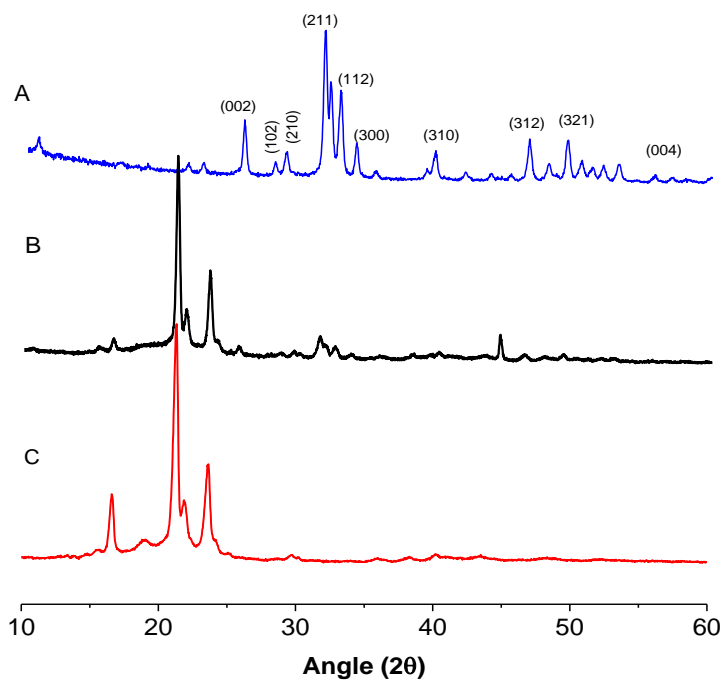


Figure S8. XRD patterns of A) pure NHA powder, B) PHIPE-5-3 and C) PHIPE-10-0

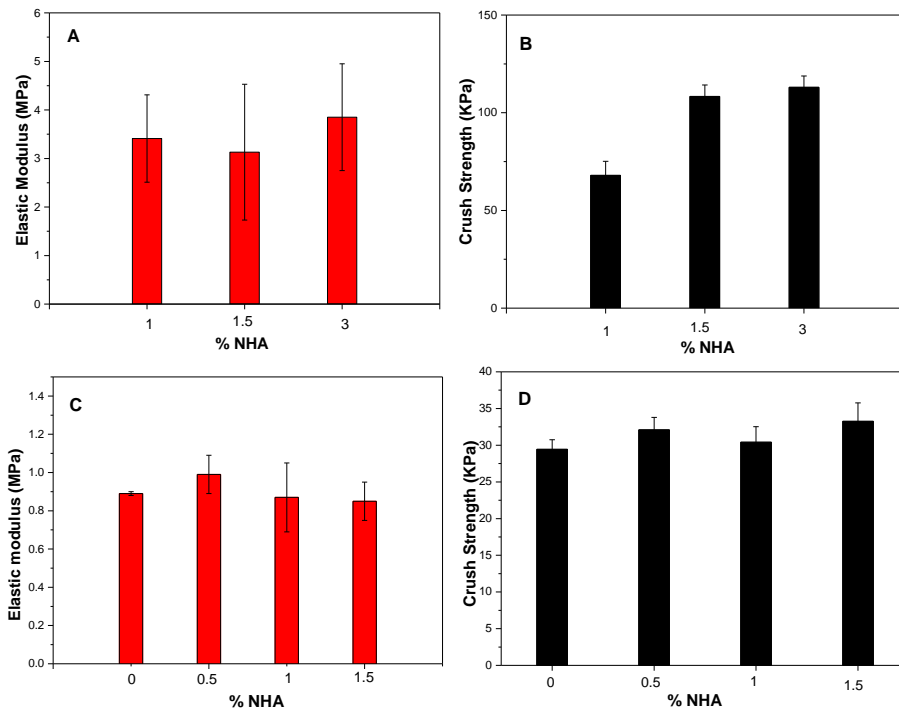


Figure S9. PolyHIPEs elastic modulus as a function of NHA concentration of A) PHIPE-5-Y and C) PHIPE-10-Y, and PolyHIPEs crush strength B) PHIPE-5-Y and D) PHIPE-10-Y

An approximation of the total pore volume ( $V_T$ ) was estimated as  $\frac{1}{\rho_b} - \frac{1}{\rho_w}$  where  $\rho_b$  is the polyHIPE bulk density calculated by measuring the volume of monoliths with regular shape and  $\rho_w$  is the wall density that corresponded to the density of PLLA/PCL blend that is ca.  $0.9091 \text{ g cm}^{-3}$  according with our previous work<sup>1</sup>. The amount of NHA is much smaller than the polymer blend, thus the density of the polymer blend was only considered as the pore wall density. The results indicate that  $V_T$  values increase as the amount of surfactant increase, accordingly with previous works reported (Table 1S).<sup>2,3</sup> Thus,  $V_T$  values are smaller when 5% F127 was employed in HIPEs

formulation. It has also reported that  $V_T$  has an important influence on compressive properties, since elastic modulus and crush strength increase as  $V_T$  decreases.<sup>3</sup> Compression Analysis (Table 1 and Figure S9) revealed that PolyHIPEs formulated with 5 wt% of surfactant achieved higher values of elastic modulus and crush strength than those formulated with 10 wt%, which is strongly related with the  $V_T$  values. Furthermore, the addition of NHA can improve mechanical properties,<sup>4,5</sup> as was observed for PHIPE-5-3 (Table 1 and Figure S9).

Table S1. Bulk density ( $\rho_b$ ) and total pore volume ( $V_T$ ) of PolyHIPEs.

Code PHIPE Y-Z*	$\rho_b$ (g cm <sup>-3</sup> )	$V_T$ (cm <sup>3</sup> g <sup>-1</sup> )
5-1	0.27	2.60
5-1.5	0.26	2.74
5-3	0.26	2.74
10-0	0.22	3.44
10-0.5	0.21	3.66
10-1	0.22	3.44
10-1.5	0.22	3.44

\*Y and Z are the concentrations of surfactant and NHA, respectively, employed in HIPEs formulation.

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