

Electronic Supplementary Information (ESI)

Two-photon Microscopy as a visual tool for polymer compatibilization monitoring: The PE-EVOH case.

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Methods

General. *Nuclear magnetic resonance (NMR).* NMR spectra were recorded on a Bruker Advance 400 (400 MHz). Chemical shifts are reported in parts per million (ppm). ¹H-NMR chemical shifts are given in reference to the residual solvent peak (2.50 ppm for deuterated dimethyl sulfoxide (DMSO-d₆) and 6.00 ppm for deuterated 1,1,2,2-Tetrachloroethane (TCE-d₂). High temperature NMR spectra were recorded at 80°C in TCE-d₂.

Infrared spectroscopy (IR). Infrared absorption spectroscopy analyses were performed with a Perkin Elmer FT-IR Spectrum Two instrument in the mode of Attenuated Total Reflectance (ATR) using powder samples. The background was subtracted from every spectrum recorded.

Elemental Analysis (EA). Elemental analyses were recorded on a CHNS Thermo Fisher FlashSmart. The nitrogen content was determined by means of thermal conductivity (TC) and volumetric analysis.

Differential scanning calorimetry (DSC): Melting temperatures (T_m) and enthalpies of the transition (ΔH_m) were measured by differential scanning calorimetry (DSC) using a DSC6000 from Perkin Elmer. DSC measurements were carried out under nitrogen between 0 and 200 °C at a scan rate of 10 °C min⁻¹. The sample weight was in the range 5-10 mg. The transitions were measured in the second heating curves.

Compression molding. Films of the samples were obtained by compression molding the polymers between Teflon plates, with an appropriate spacer, at 180 °C for 1 min under a pressure of 2 ton m⁻² (Carver C12, laboratory press).

Dynamic mechanical analysis (DMTA). Rectangular samples suitable for DMTA were cut to dimension of 3 x 5 x 0.5 mm (length x width x thickness). Samples were measured on a TA Instruments Q800 in tensile mode. The E', E'' and Tanδ were monitored while screening the samples during a temperature sweep from -100 °C to 150 °C at 3 °C min⁻¹. An oscillation frequency of 1 Hz with an oscillation amplitude of 10 μm were applied.

Two-photon microscopy (2PM): The polymer specimens were characterized with a Two-Photon Microscope Nikon A1R MP+ Upright supplied with a femtosecond pulsed laser Coherent Chameleon Discovery (~ 100 fs pulse duration with 80 MHz repetition rate, tunable wavelength output 660-1320 nm). To focus the excitation beam and to collect the two-photon excited fluorescence (TPEF) signal, a 25x water dipping objective with numerical aperture (N.A.) 1.1 and working distance 2.0 mm was used. Due to the characteristics of the samples (thin films) we decided to collect images in air (instead of water). This choice affects the N.A. (which becomes lower), and as a consequence the resolution. In fact, according to the Rayleigh criterion, in multiphoton microscopy the lateral resolution (x-y) is $d = \frac{0.61\lambda}{N.A.}$, while the axial resolution is $z = \frac{2n\lambda}{N.A.^2}$, where λ is the excitation wavelength, n the medium the refractive index, and $N.A.$ the numerical aperture.¹

TPEF signal was directed by a dichroic mirror to a series of three non-descanned detectors (high sensitivity GaAsP photomultiplier tubes) allowing fast image acquisition. Optical filters preceded the detectors allowing the simultaneous acquisition of three separated channels: blue channel (415-485 nm), green channel (506-593 nm), and red channel (604-679 nm). The operation software of the microscope performed the overlay and the processing of the three

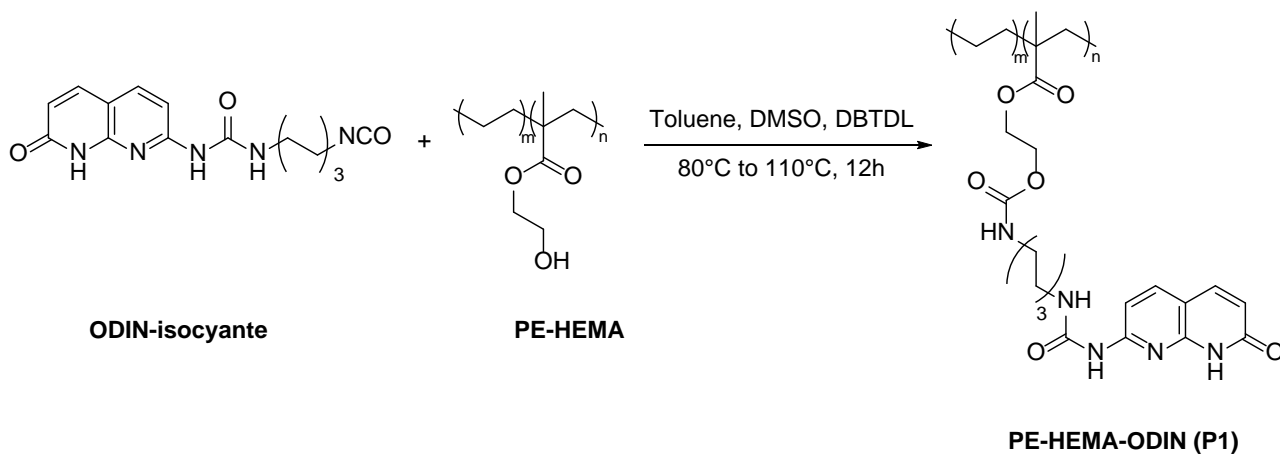
¹ M. D. Young, J. J. Field, K. E. Sheetz, R. A. Bartels, J. Squier, *Adv Opt Photonics* **2015**, 7, 276–378.

channels images. A fourth photomultiplier GaAsP detector, connected to the microscope through an optical fiber and preceded by a dispersive element, was used to record emission spectra of the two-photon excited samples (wavelength range 400-630 nm with a bandwidth of 10 nm).

Z-stack acquisitions were collected with 0.5 μm steps and images with a field of view of 500x500 μm (1024x1024 pixel) and a 2.2 s dwell time. During Z-stack acquisitions, several 2D images of the sample are collected at different depths (in this case one image every 0.5 μm) and then processed by the microscope software to obtain a 3D reconstruction of the sample.

Synthesis. Unless otherwise specified, chemicals and solvents were purchased from Sigma-Aldrich and used as received. Poly(ethylene-co-(2-hydroxyethyl methacrylate) (PE-HEMA, 2.1% mol of HEMA) was provided by SABIC. Co-polymer Ethylene Vinyl-Alcohol (EVOH, 32 mol% of ethylene) was purchased from EVALTM. The synthetic procedures for ODIN isocyanate 2 and PE-HEMA-ODIN (P1) were adapted from literature⁸ (Schemes S1 and S2).

Synthesis of PEHEMA-ODIN (P1):



Scheme S2: Functionalization of PE-HEMA with ODIN-isocyanate

50 mL of dry toluene were inserted into an anhydrous Schlenk equipped with a magnetic stirrer. The solvent was degassed through three cycles of freeze-pump-thaw, then PE-HEMA (1 g) was added under nitrogen and dissolved under stirring at 80 °C for 1 h. Once a clear solution was obtained, ODIN-isocyanate(400 mg) was added under nitrogen as a solid, followed by addition of few drops of DBTDL as catalyst and 5 mL of dry DMSO to promote the dissolution of ODIN-isocyanate. The temperature of the mixture was raised to 110 °C and stirred for 12 hrs. The solution was cooled to room temperature and isopropanol was added to promote the precipitation of the functionalized polymer. The yellow product was then filtered under vacuum, washed with isopropanol, and dried in a vacuum oven at 40°C for 12 hrs.

¹H NMR (400 MHz, TCE-d₂, 80°C): δ(ppm) = 0.85-2.00 (m, PE backbone), 3.24 (m, 2H), 3.40 (s, 2H), 3.86 (t, J=4.8 Hz, 2H), 4.26 (t, J=4.8 Hz, 2H), 4.30 (s, 6H), 6.61-7.89 (m, 4H), 8.23 (s, 1H), 11.09 (s, 1H), 12.71 (s, 1H).

IR, $\tilde{\nu}$ (cm⁻¹): 3406 (N-H stretching), 2916 (C-H stretching), 2849 (C-H stretching), 1729 (C=O stretching, HEMA), 1660 (C=O stretching, ODIN), 1595 (N-H bending), 1532 (C-N stretching), 1472 (C-H bending), 1234 (C-O stretching, ODIN), 1138 (C-O stretching, HEMA).

Elemental Analysis, the degree of functionalization of PE-HEMA is 1.4 mol% calculated by the content of nitrogen (C 78.57%, H 12.83%, N 2.82%).

¹H-NMR

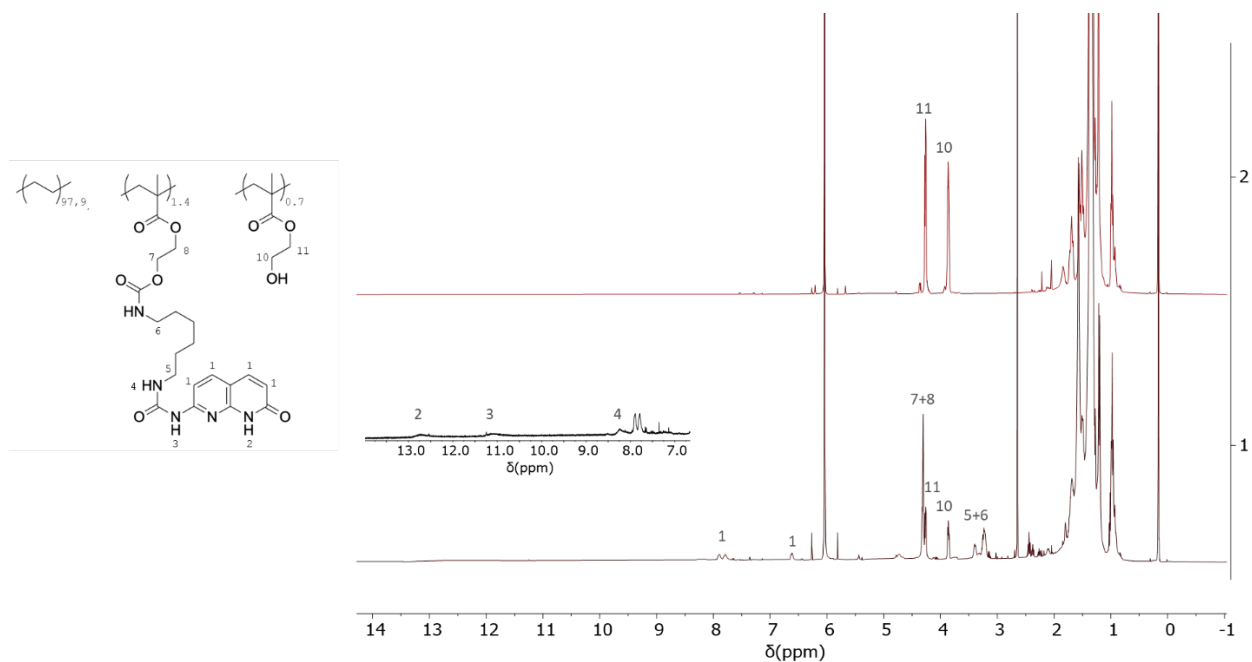


Figure S1: ¹H-NMR spectra of **P1** (spectrum below) and PE-HEMA (spectrum above). In the upper part of the figure the magnification (between 6 and 13 ppm) of the spectrum of **P1** is reported to show the signals related to the hydrogen bonding.

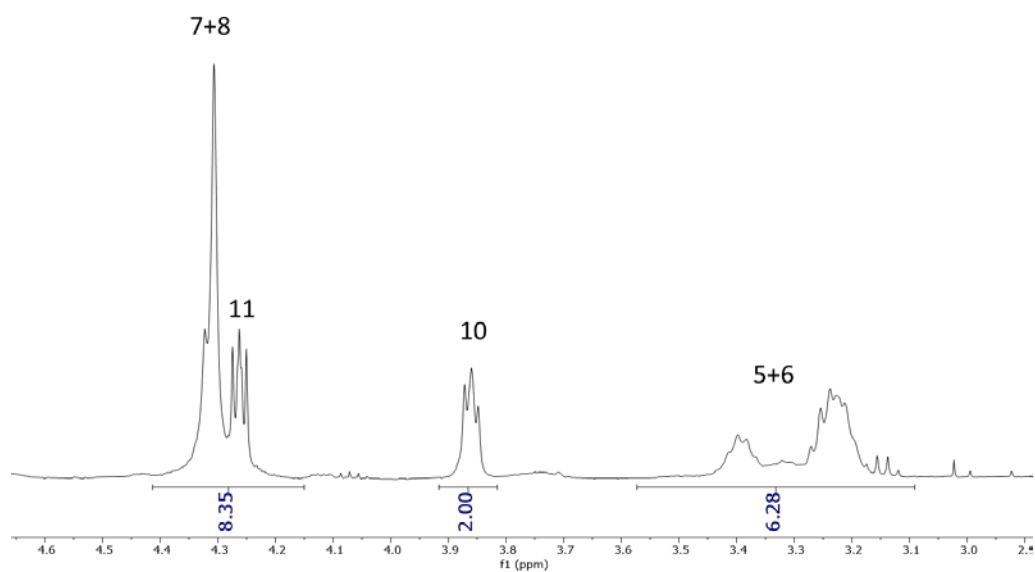


Figure S2: magnification (between 2.9 and 4.6 ppm) of the ¹H-NMR spectrum of **P1** is reported.

General procedure for the formation of the blends between EVOH and PE-HEMA-ODIN (P1). Compound P1 was dissolved in o-dichlorobenzene (ODCB, 1 mg/mL) at 110°C, while EVOH was dissolved in DMSO (25 mg/mL) at the same temperature (Scheme 1). Once the dissolution of both polymers was reached, the solution of EVOH was slowly added to the solution of P1, and the resulting solution was stirred at 110°C for 3 hrs. Subsequently, the solution was cooled to room temperature and 50 mL of isopropanol were added. The obtained precipitate was filtered, washed with isopropanol and dried under vacuum at 60°C for 72 hrs. The blends were characterized by elemental analysis (EA) (see Table 1), differential scanning calorimetry (DSC) and dynamic mechanical thermal analysis (DMTA) (see Table 2 and Figure S7, S8, S9 and S17).

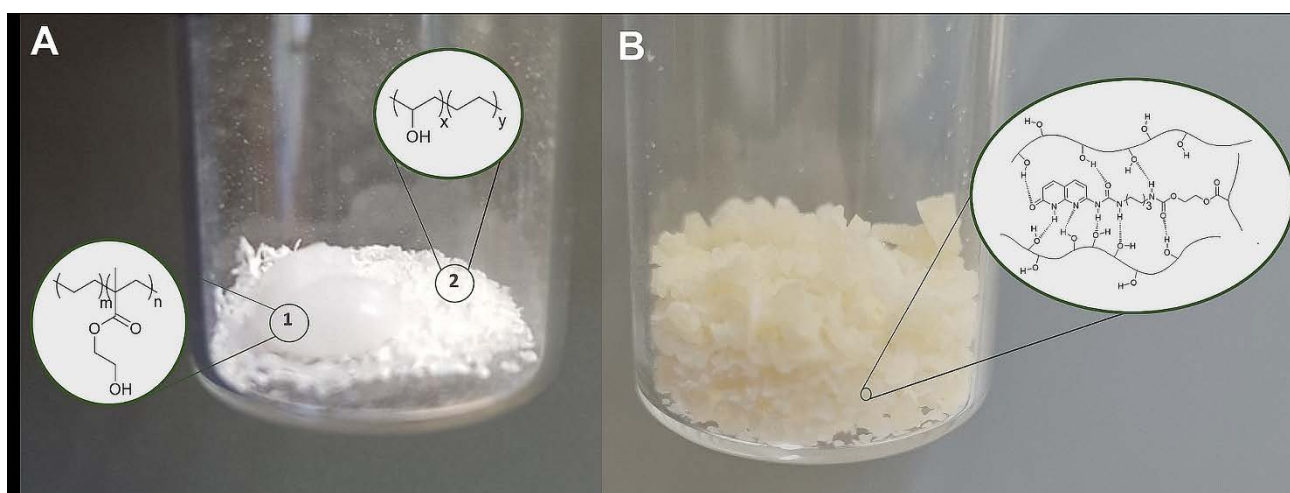


Fig. S3 (A) EVOH/PE-HEMA mixture, (B) **B2** blend composed by EVOH/P1 1:1 w/w.



Fig. S4. Images providing the transparency of the films obtained by compression molding.

ATR

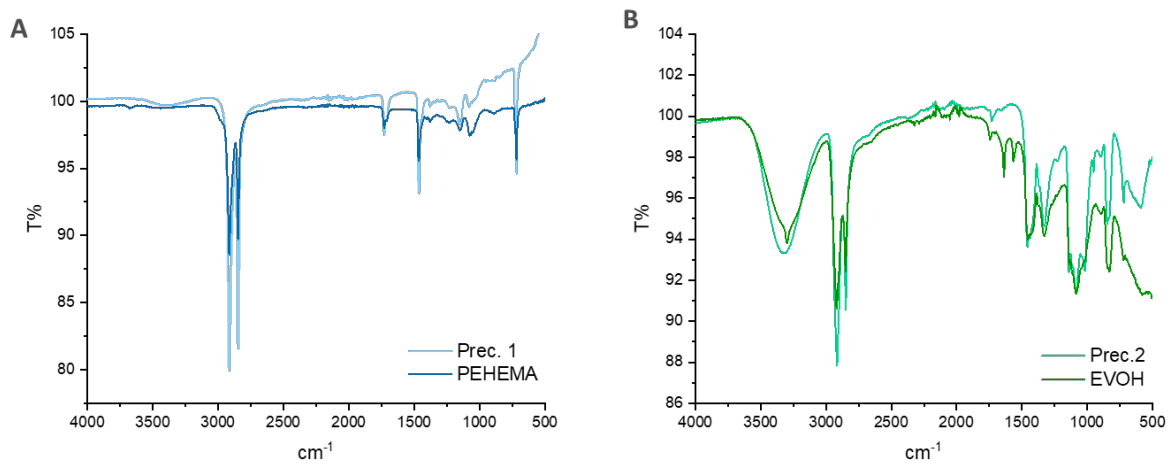


Figure S5: ATR spectra of (A) precipitate 1 and PE-HEMA polymer and (B) precipitate 2 and EVOH polymer

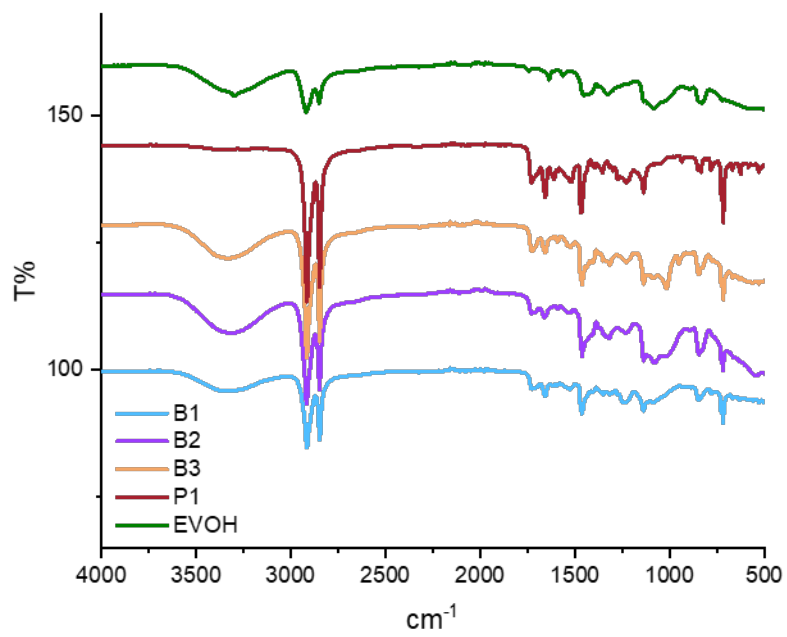


Figure S6: ATR spectra of **B1**, **B2**, **B3**, **P1** and EVOH.

DSC

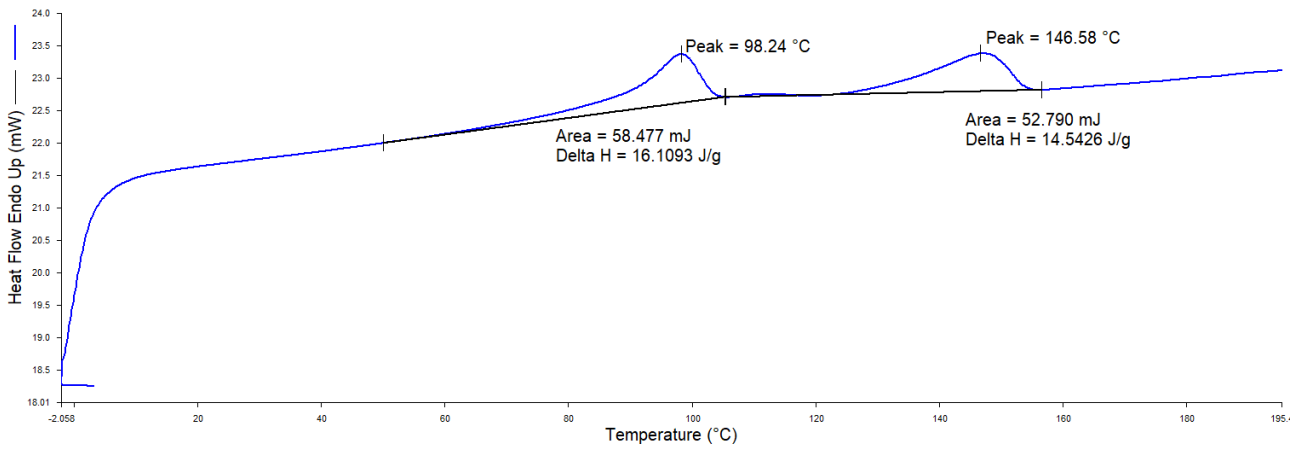


Figure S7: DSC thermographs of **B1**.

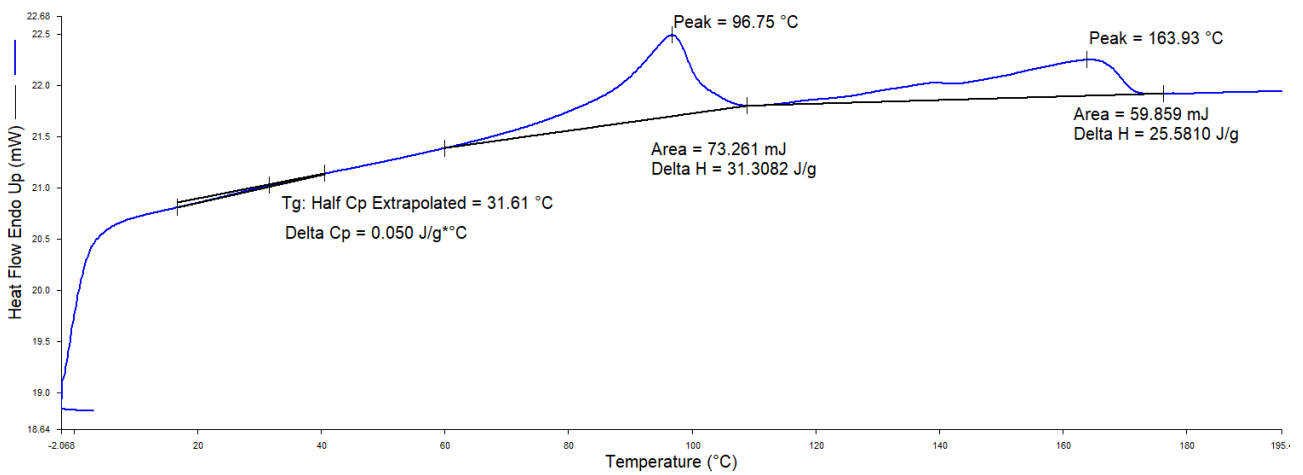


Figure S8: DSC thermographs of **B2**.

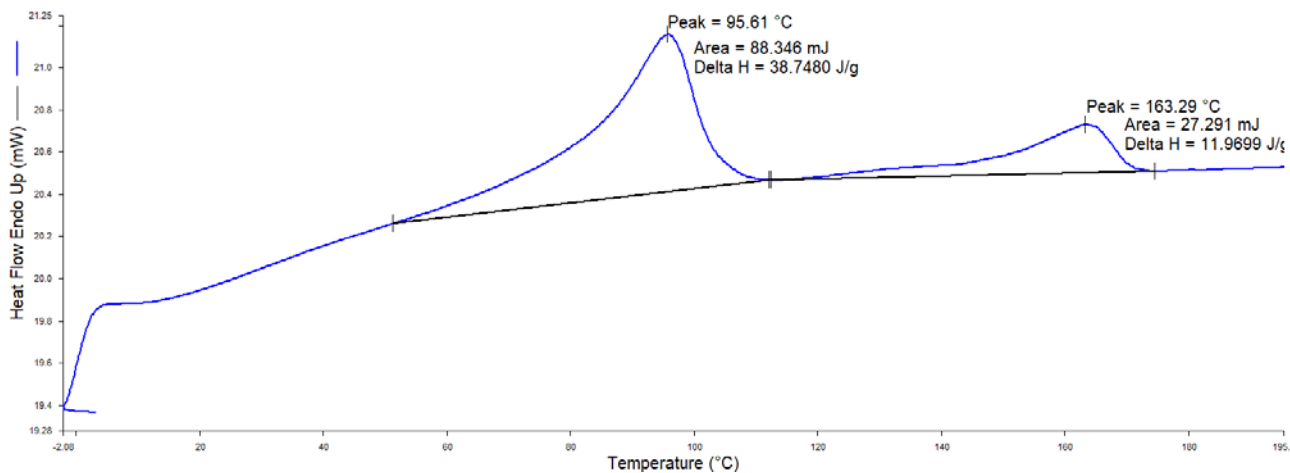


Figure S9: DSC thermographs of **B3**.

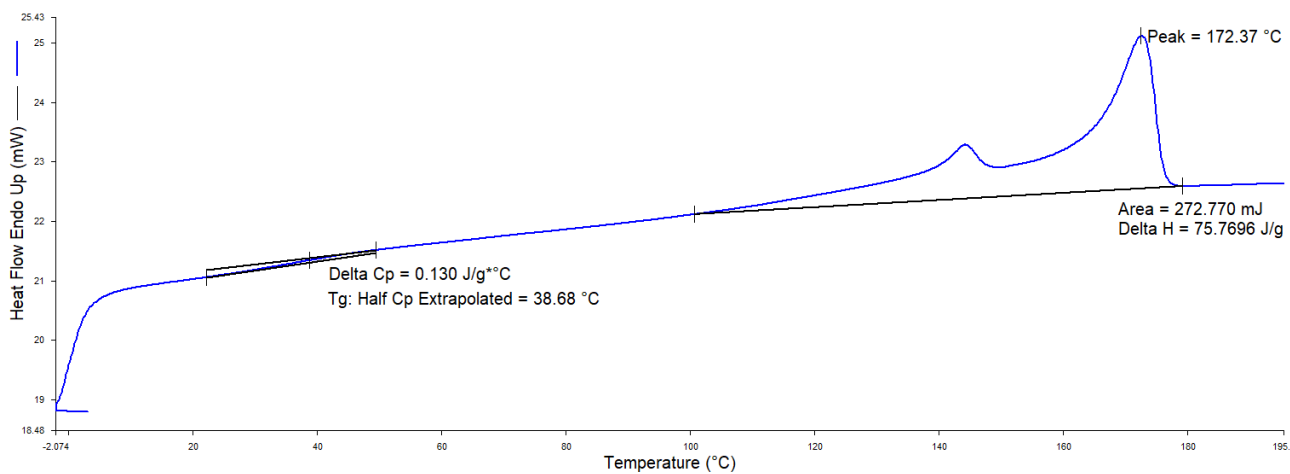


Figure S10: DSC thermographs of **EVOH**.

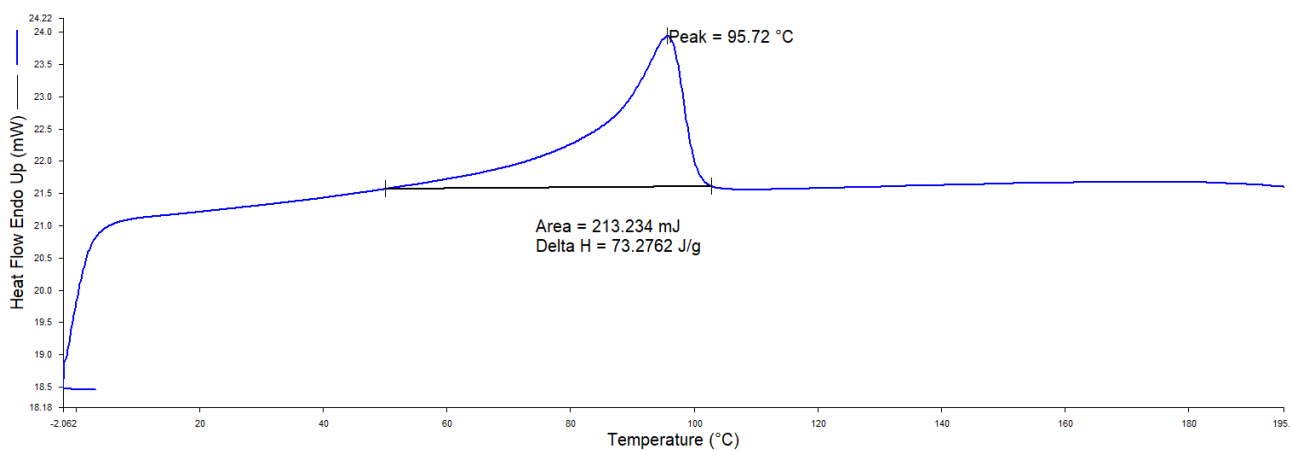


Figure S11: DSC curve of **P1**.

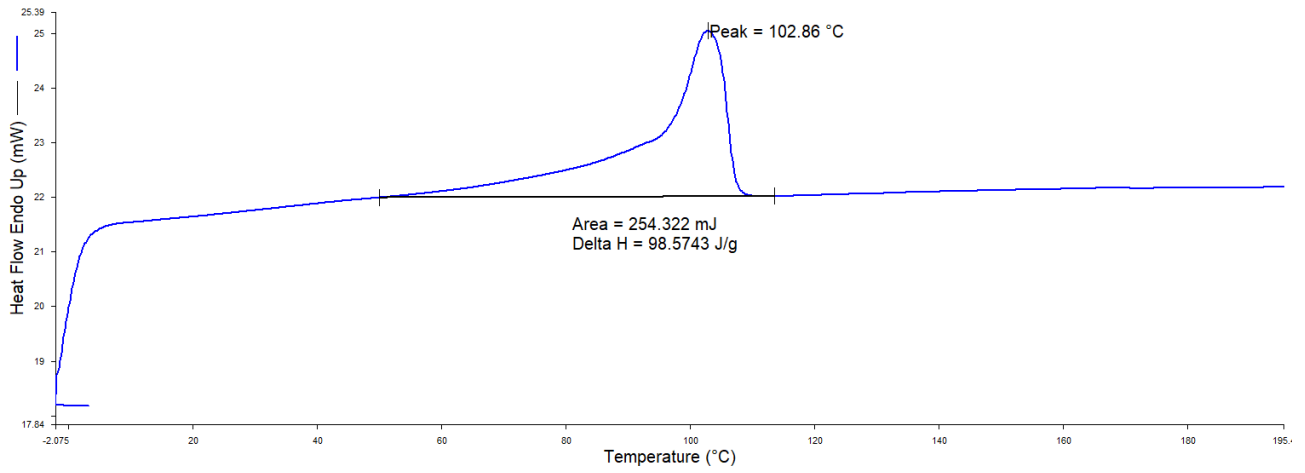


Figure S12: DSC curve of PE-HEMA.

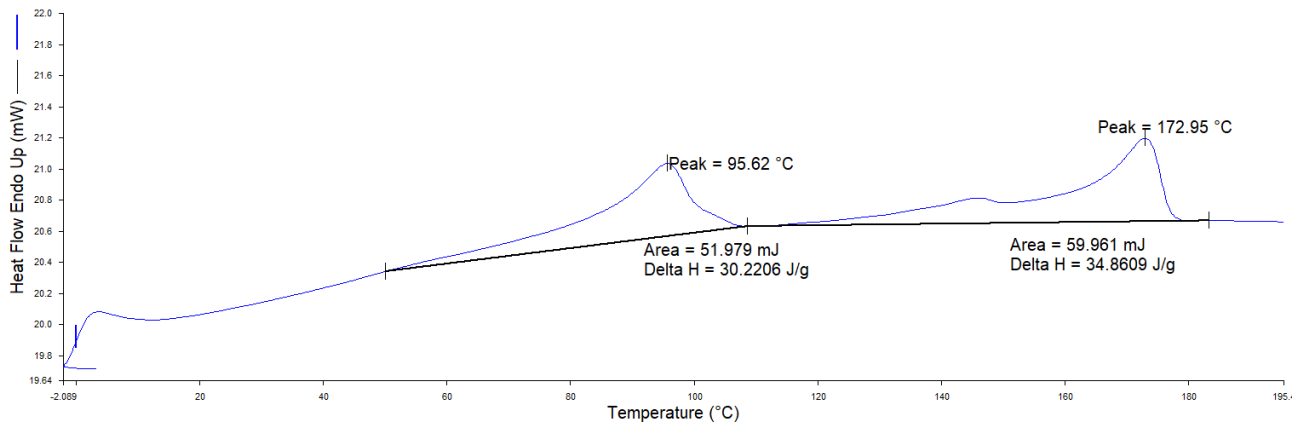


Figure S13: DSC curve of B2-1.

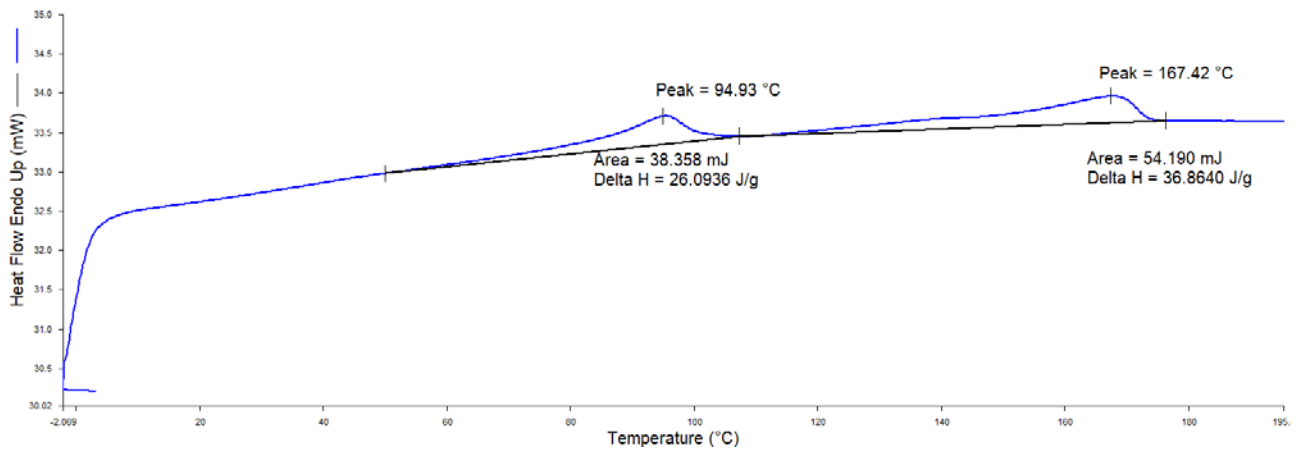


Figure S14: DSC curve of B2-2.

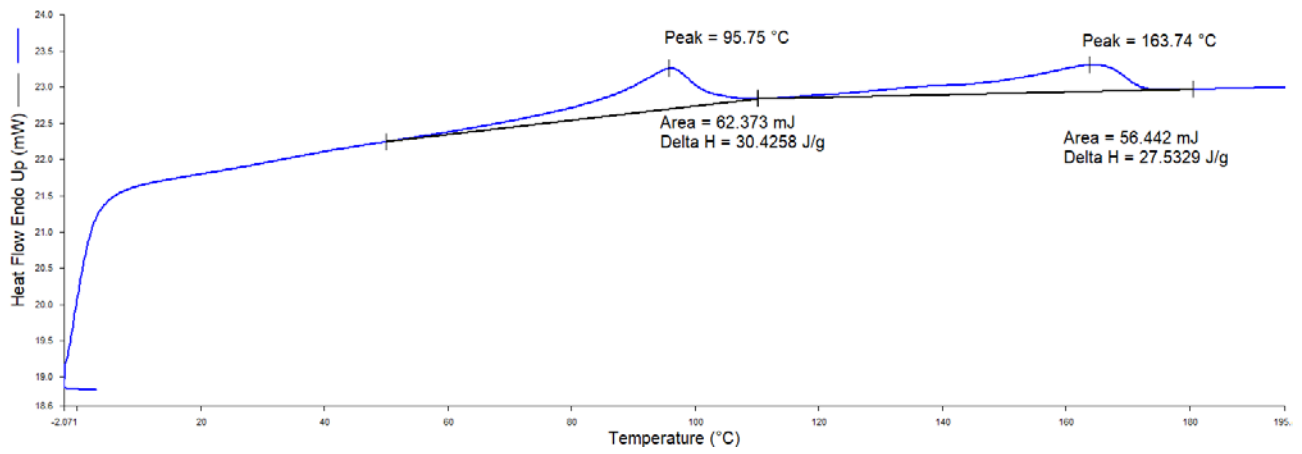


Figure S15: DSC curve of **B2-3**.

DMTA

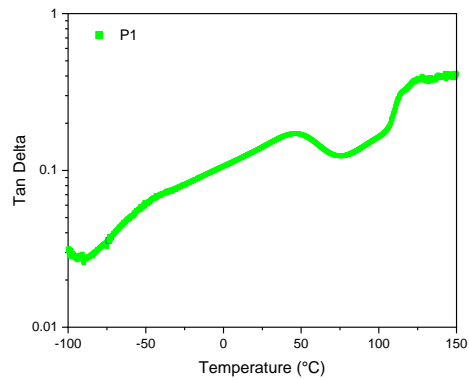


Figure S16: DMTA thermogram of **P1** shows the crosslinking effect of PEHEMA after the melting temperature.

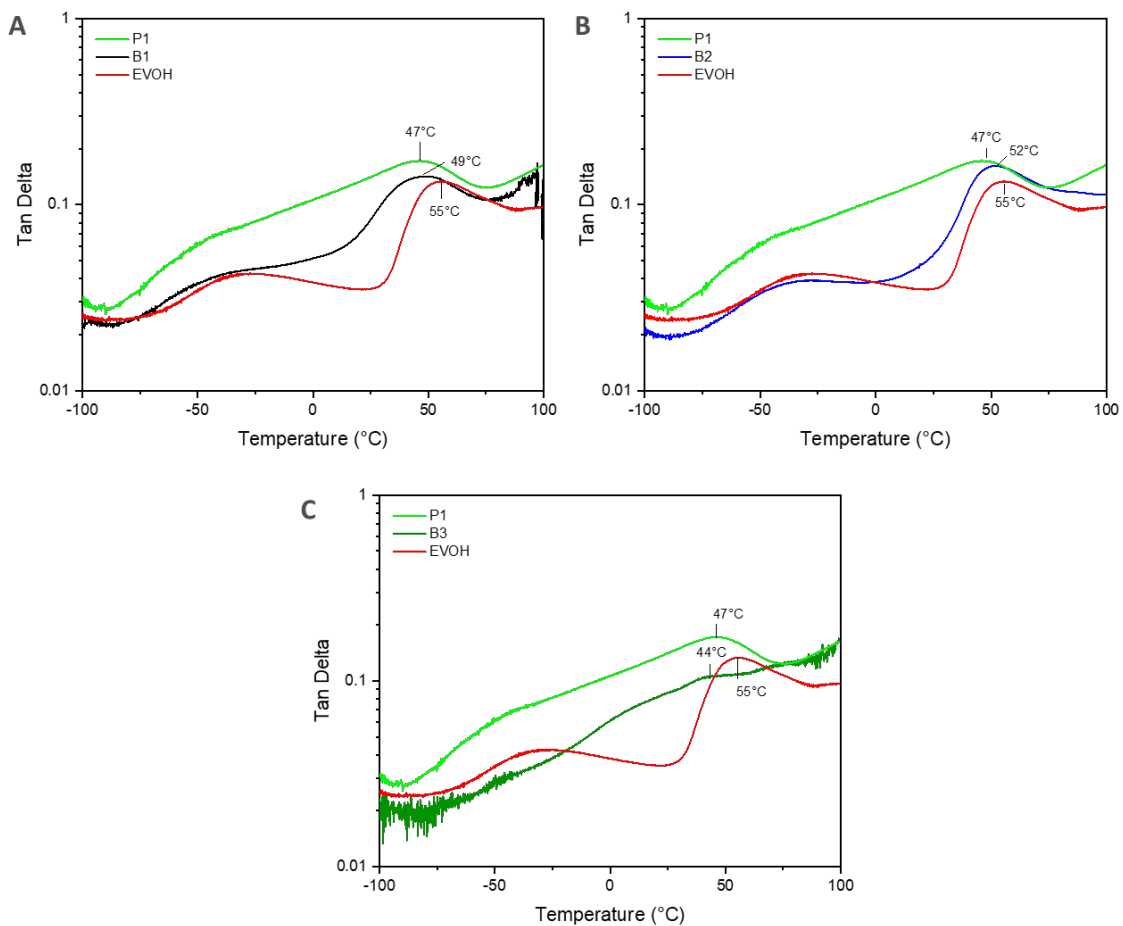


Figure S17: DMTA thermograms of (A) **B1** (black curve), (B) **B2** (blue curve) and (C) **B3** (dark green curve) using **P1** (light green curve) and EVOH (red curve) as reference in each figure to monitor the T_{β} of the polymer blends.

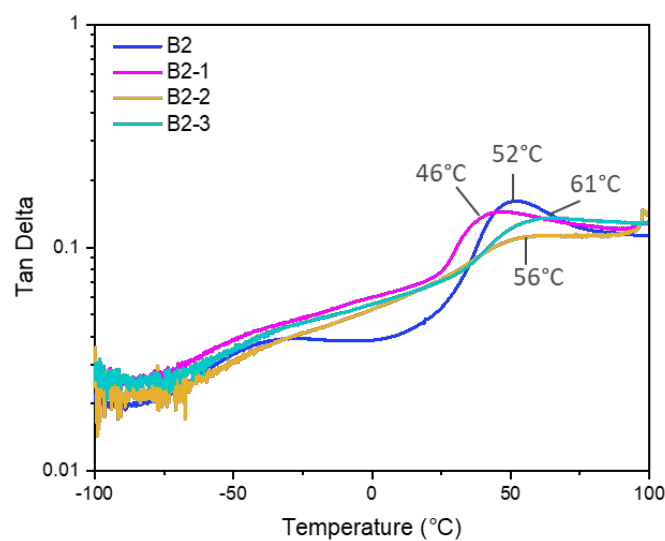


Figure S18: DMTA thermograms of **B2** (blue curve), **B2-1** (pink curve), **B2-2** (ochre curve) and **B2-3** (light blue curve) to monitor the T_{β} of the polymer blend after reprocessing.

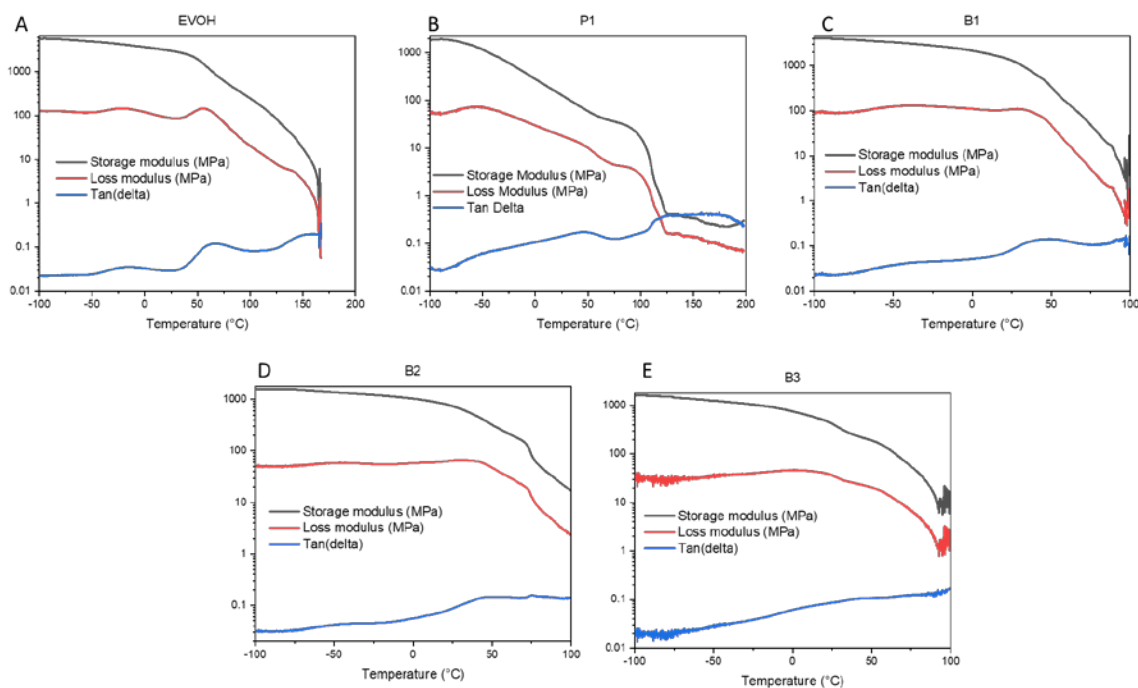


Figure S19: DMTA thermograms of (A) EVOH, (B) **P1**, (C) **B1**, (D) **B2**, (E) **B3**. Black curve: storage modulus, red curve: loss modulus, blue curve: $\text{Tan}\delta$.

Two-photon microscopy

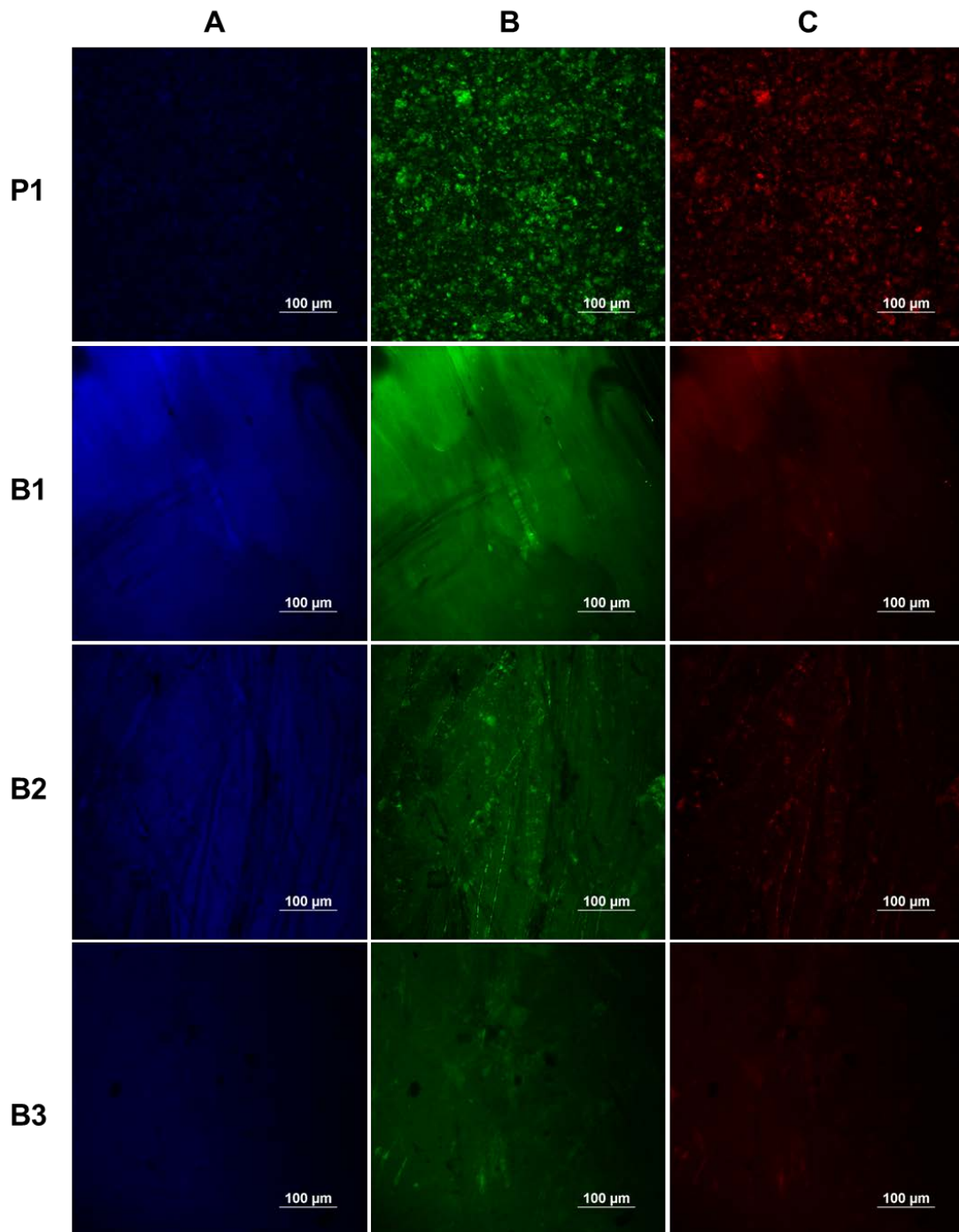


Figure S20: Images acquired on the surface of specimens **P1**, **B1**, **B2** and **B3**. Panels A: blue channel; panels B: green channel; panels C: red channel. All images were collected exciting the samples at 820 nm.