Supplementary material for:

Synthesis and characterization of poly(ester amide)-based materials for 3D printing of tissue engineering scaffolds

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Materials and Methods

Rheological Properties

The rheological properties of the AAA-PEAs and PCL were studied using a Discovery Hybrid rheometer-2 (TA Instruments, USA) apparatus with parallel plates (diameter: 25 mm, gap: 350μm). Hot-pressed films, with a diameter of 25 mm, were analyzed to guarantee that before the test all the samples were subjected to the same thermal treatment. Melt complex viscosity as a function of temperature was measured by applying an oscillatory strain up to 1 %, at a frequency of 1 rad/s, while continuously decreasing the temperature at a rate of 5 °C min, from 100 °C to 18 °C.

Supporting Figures

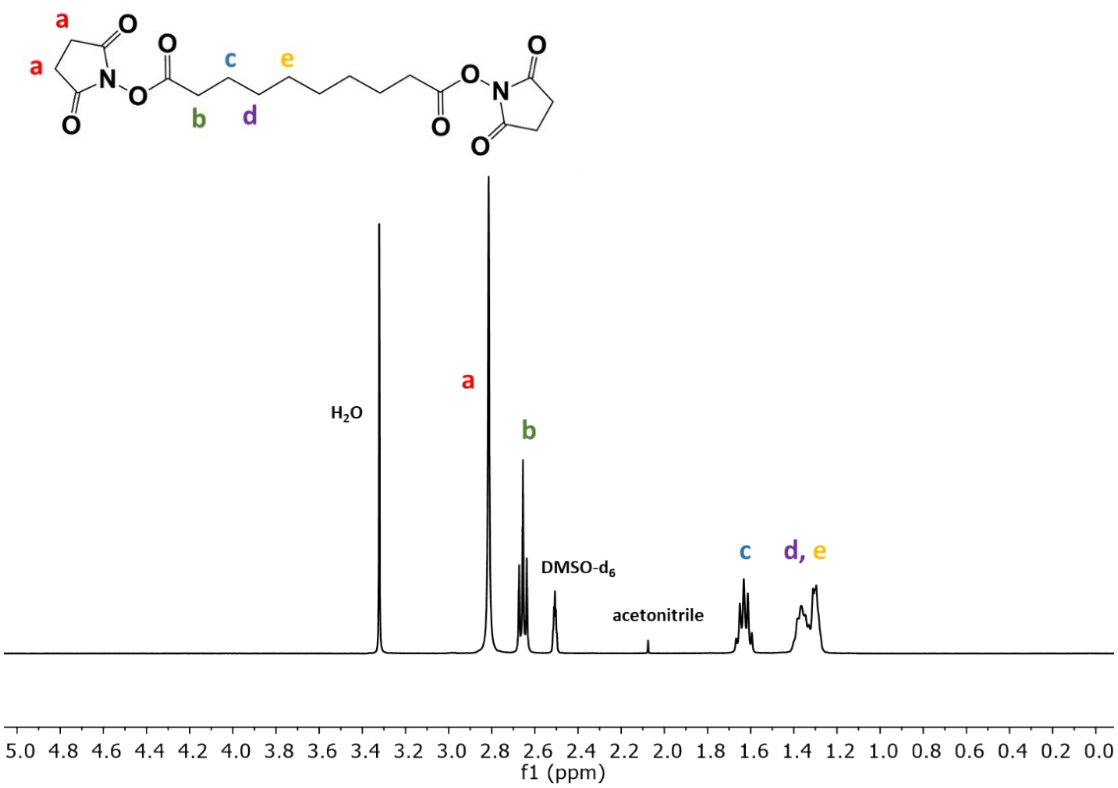

Figure S1. *^{1}H-NMR spectrum of activated diester of sebacoyl chloride.*

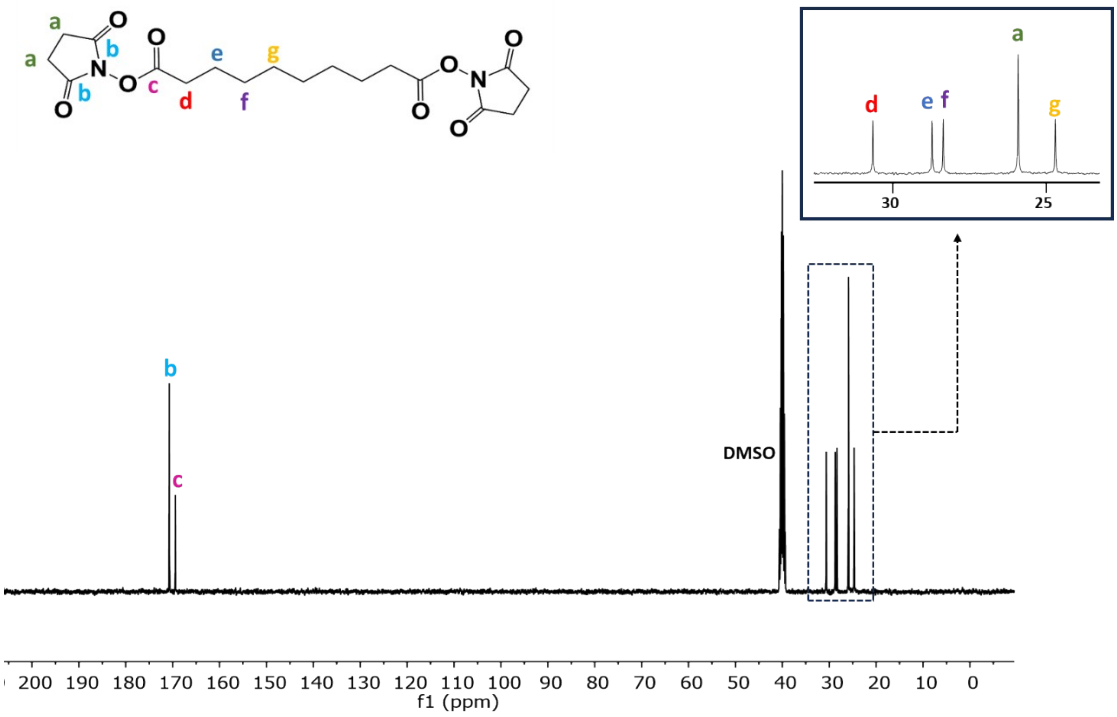

Figure S2. *^{13}C-NMR spectrum of activated diester of sebacoyl chloride.*

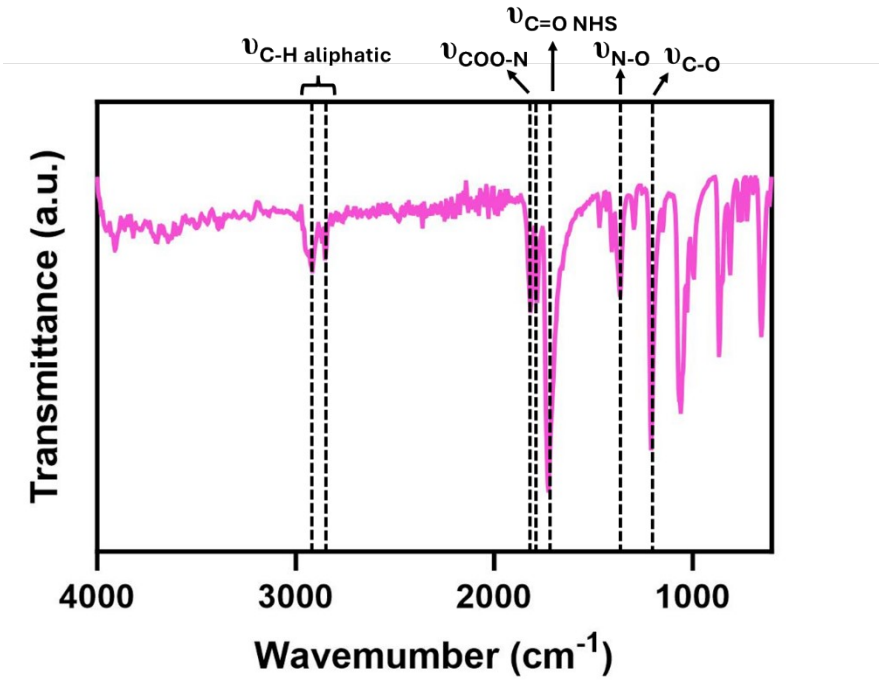

Figure S3. *FTIR spectra the synthesized activated diester of sebacoyl chloride.*

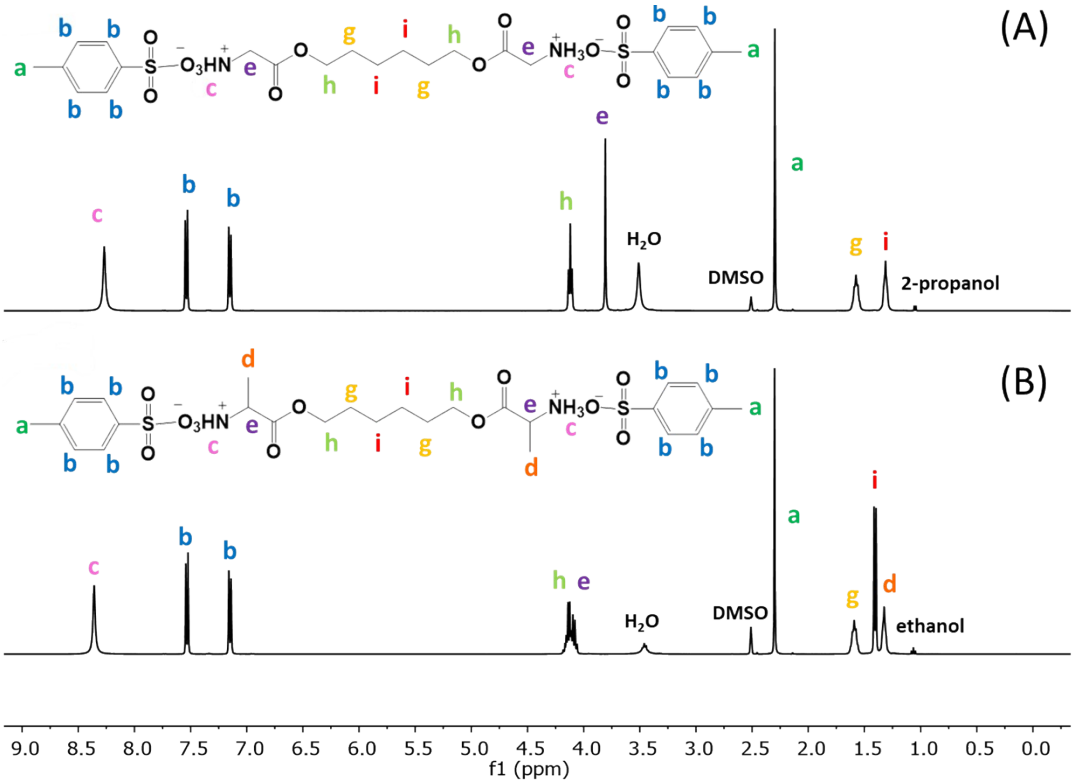

Figure S4. *[1]H-NMR spectrum of (A) di-p-toluenesulfonic acid salt of bis(glycine)-hexane 1,6-diester monomer (BAAD-gly) and of (B) di-p-toluenesulfonic acid salt of bis (L-alanine)-hexane 1,6-diester monomer (BAAD-ala).*

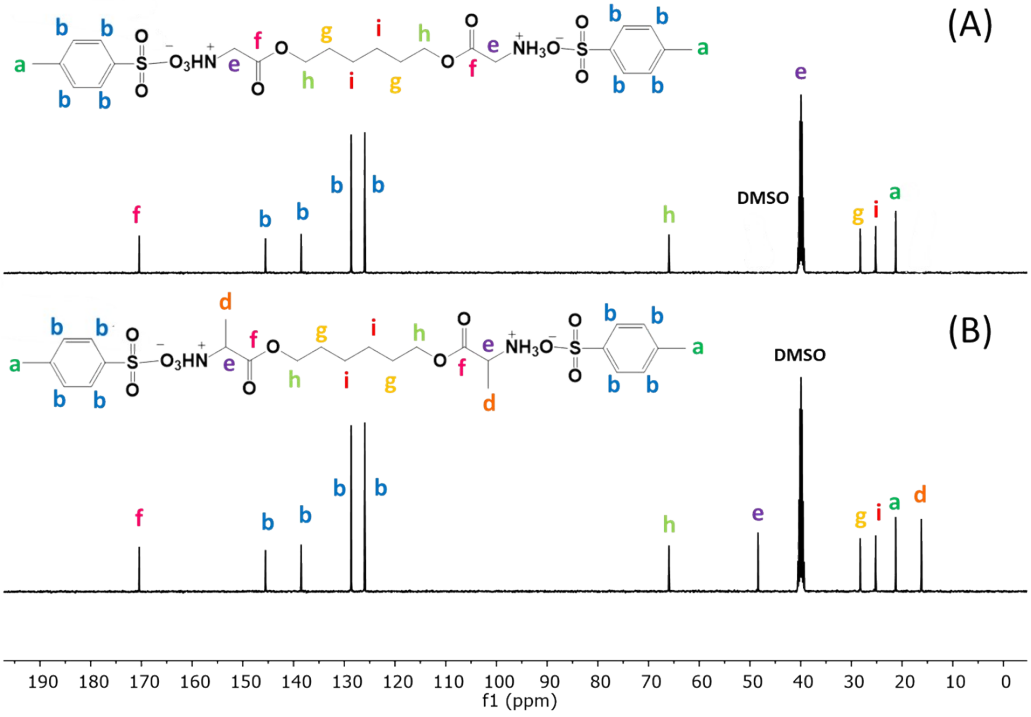

Figure S5. *[13]C-NMR spectrum of di-p-toluenesulfonic acid salt of bis(glycine)-hexane 1,6-diester monomer (BAAD-gly) and of di-p-toluenesulfonic acid salt of bis (L-alanine)-hexane 1,6-diester monomer (BAAD-ala).*

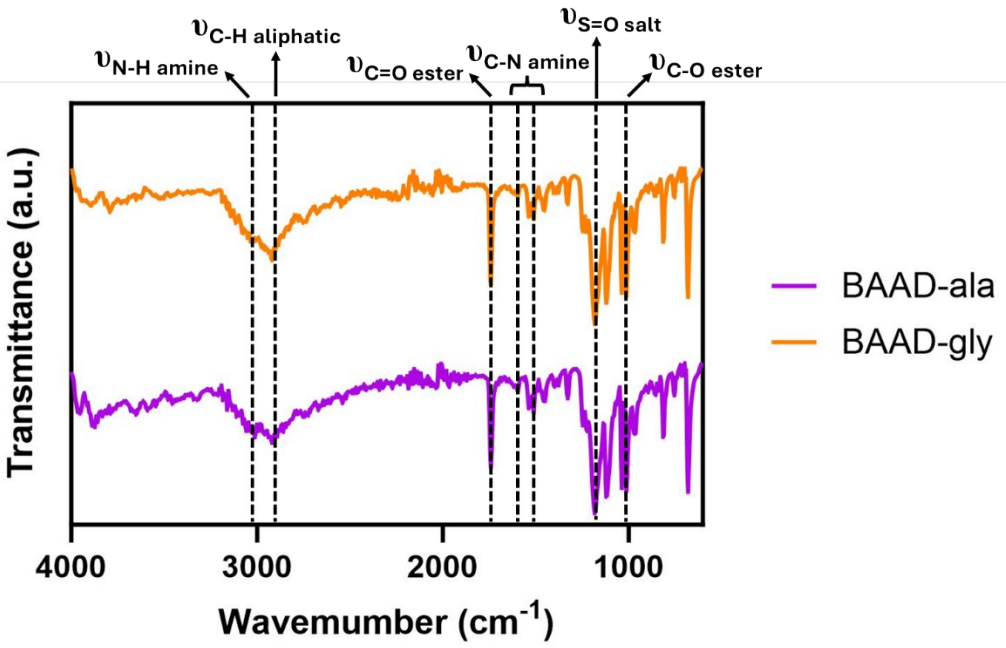

Figure S6. *FTIR spectra the synthesized BAADs.*

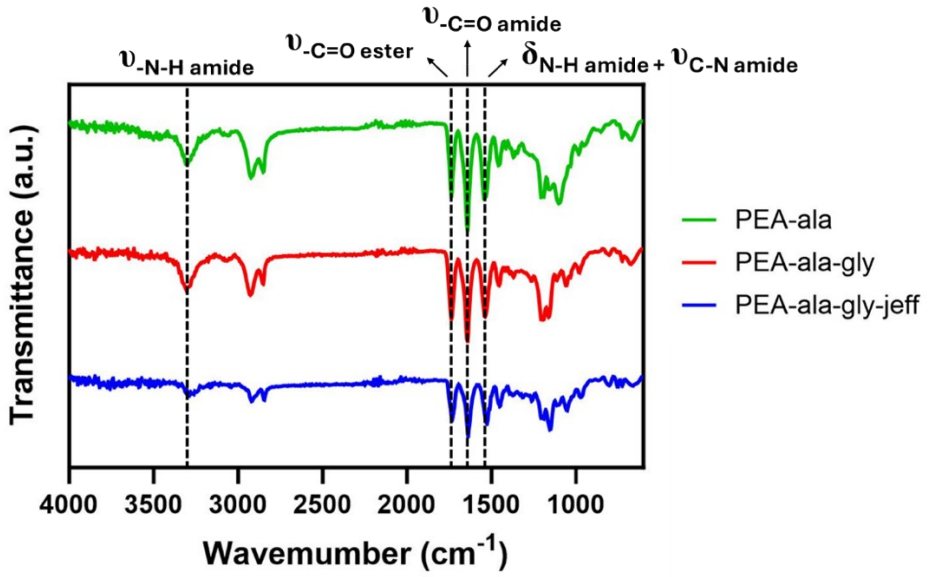

Figure S7. *FTIR spectra of the synthesized AAA-PEAs.*

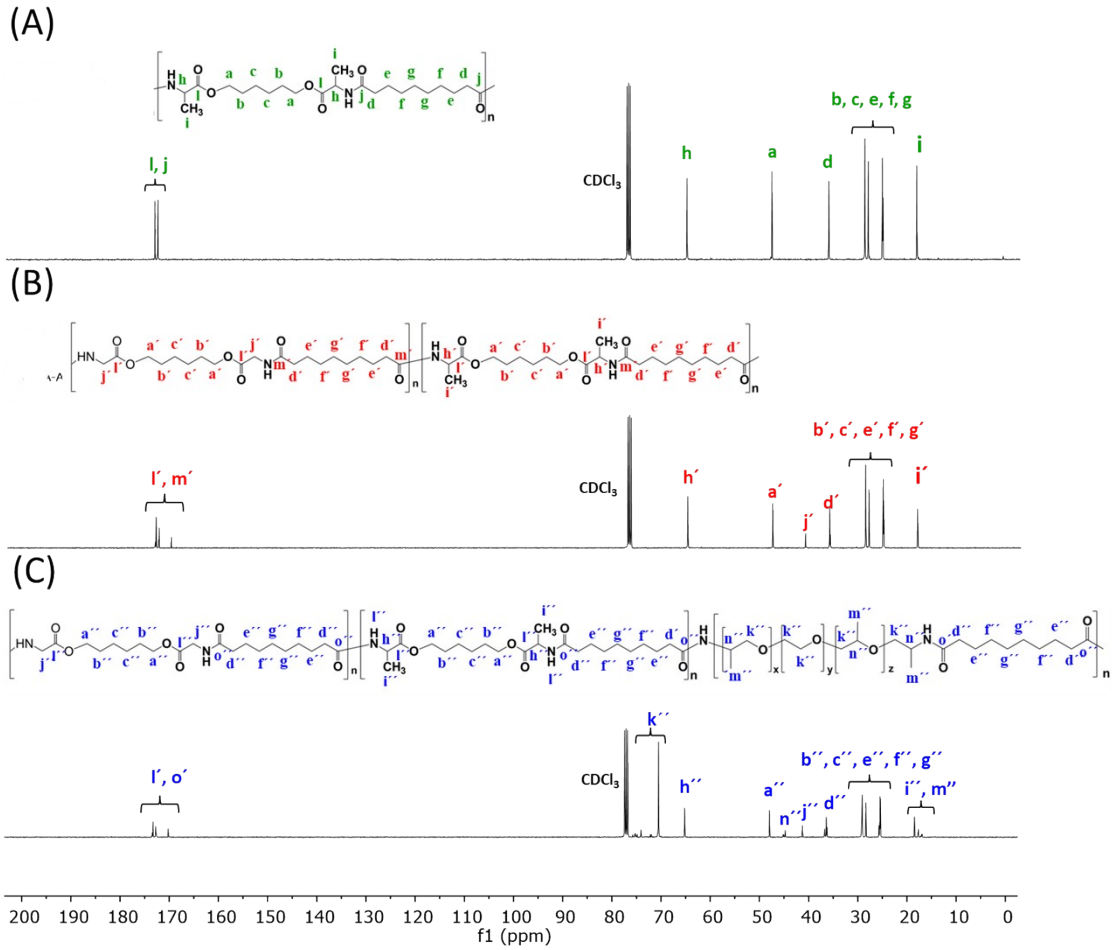

Figure S8. *^{13}C NMR spectra of (A) PEA-ala, (B) PEA-gly-ala and (C) PEA-ala-gly-jeff.*

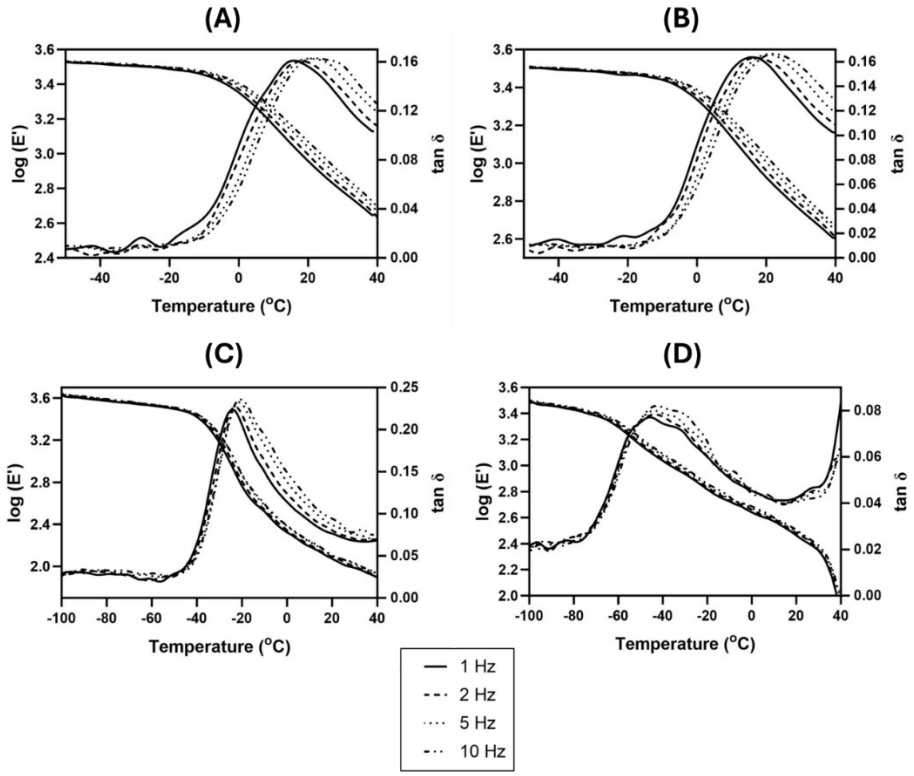

Figure S9. *DMTA traces of the scaffolds, in multifrequency mode: PEA-ala (A); PEA-ala-gly (B); PEA-ala-gly-jeff (C); PCL (D).*

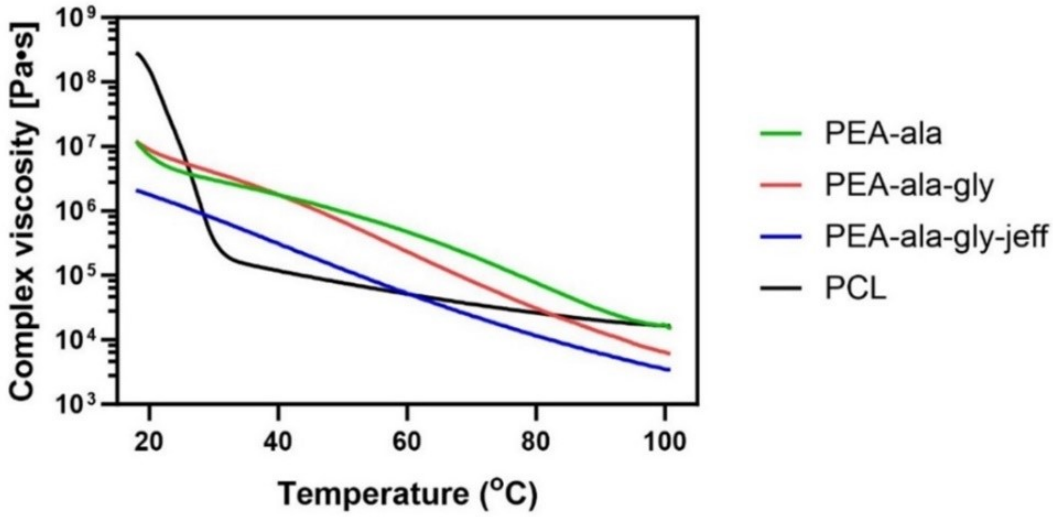

Figure S10. Complex viscosity of AAA-PEAs and PCL as a function of temperature.

Supporting Tables

Table S1. *Quantities of reactants and solvent used in the synthesis of the AAA-PEAs.*

Sample	n (BAAD-gly) (mmol)	n (BAAD-ala) (mmol)	n (jeff) (mmol)	n (triethylamine) (mmol)	n (activated dieste) (mmol)	V (DMSO) (mL)
PEA-ala	0	6	0	13	6	10
PEA-ala-gly (75:25)	1.5	4.5	0			
PEA-ala-gly-jeff (50:25:25)	3	1.5	1.5			

Table S2. *Optimised set of process parameters used for the printing of PCL and AAA-PEAs scaffolds. Parameters are denoted as deposition velocity (DV), slice thickness (ST), liquefier temperature (LT), extrusion pressure (EP) and screw rotation velocity (SRV).*

Sample	DV (mm/s)	ST (µm)	LT (°C)	EP (MPa)	SRV (rpm)
PEA-ala	10	200	100	0.2	18
PEA-ala-gly (75:25)	10	200	100	0.2	18
PEA-ala-gly-jeff (50:25:25)	8	200	85	0.2	18
PCL	8	200	80	0.2	18

Table S3. *Initial and final molar ratio of BAAD-ala, BAAD-gly and jeff in AAA-PEA copolymers.*

Sample	Composition	In feed molar ratio (%)	Final Molar Ratio (%)
PEA-ala-gly	BAAD-ala:BAAD-gly	75:25	74±2:26±2
PEA-ala-gly-jeff	BAAD-ala:BAAD-gly:jeff	50:25:25	53±2:24±1:23±1

Table S4. Temperatures of interest taken from the TGA and DSC analysis. T_g – glass transition temperature; T_m – melting temperature; T_c - crystallization temperature (DSC); $T_{5\%}$ - temperature at which the sample loses 5% of its initial weight (TGA); T_p-temperature at which the rate of weight loss attains its maximum (DTG).

	1st heating		**Cooling**	**2nd heating**		**TGA**		
	T_m (°C)	**T_g (°C)**	**T_c (°C)**	**T_m (°C)**	**T_g (°C)**	**T_p (°C)**	**$T_{5\%}$ (°C)**	**Char residue (%)**
PEA-ala	83.0±0.5	-	-	-	17.8±0.1	369.3±3.4; 417.3±1.8; 458.8±3.3	337.0±5.1	4.1±0.1
PEA-ala-gly	67.7±0.6; 79.8±0.4	-	-	-	13.3±0.3	361.2±2.7; 412.4±4.1; 460.0±8.4	335.7±1.0	3.4±0.2
PEA-ala-gly-jeff	55.2±1.0; 75.3±0.5	-	-	-	-12.5±0.6	371.8±4.9; 421.4±4.0	340.7±1.3	3.5±0.2
PCL	60.0±0.5	-	30.6±1.8	56.2±0.3	-	407.9±1.6	379.6±0.5	0.61±0.1

Table S5. *Values of E' and T_g for the films, determined by DMTA.*

Films	*E*' (37°C, MPa)	T_g (°C)
PEA-ala	417.8±72,4	17.8±1.9
PEA-ala-gly	492.1±76,1	18.8±2.7
PEA-ala-gly-jeff	91.5±11,0	-22.4±3.1
PCL	-	-47.0±0.4

Table S6. *Theoretical and experimental morphological characteristics of PEAs and PCL scaffolds.*

	Theoretical values	PEA-ala	PEA-ala-gly	PEA-ala-gly-jeff	PCL
RW (µm)	330	338±16	333±5	319±10	345±28
FG (µm)	400	379±10	362±13	384±7	367±11

Table S7. *Results from micro-CT analysis performed on 3D printed scaffolds: porosity, surface area to volume ratio, and interconnectivity.*

Scaffold	Porosity (%)	Surface area to volume ratio (mm^{-1})	Interconnectivity (%)
PEA-ala	56.9	11.2	100
PEA-ala-gly	53.8	10.6	100
PEA-ala-gly-jeff	61.1	12.1	100
PCL	54.3	10.7	100

Table S8. *Enthalpy of the melting transition of PEAs and PCL after synthesis and purification and of scaffolds.*

	ΔH (J/g)	
	After synthesis and purification	**Scaffold**
PEA-ala	51.73	52.34
PEA-ala-gly	59.69	51.24
PEA-ala-gly-jeff	54.60	57.47
PCL	76.74	79.01