

Collagen / polyester-polyurethane porous scaffolds for use in meniscal repair

Gaëlle Savin ^{a,b,d}, Sylvain Caillol ^b, Audrey Bethry ^a, Eric Rondet ^c, Michel Assor ^d, Ghislain David ^b, Benjamin Nottelet ^{a,e*}

^a IBMM, Univ Montpellier, CNRS, ENSCM, Montpellier, France

gaelle.savin@enscm.fr (G.S.); audrey.bethry@umontpellier.fr (A.B.)

^b ICGM, Univ Montpellier, CNRS, ENSCM, Montpellier, France

ghislain.david@enscm.fr (G.D.); sylvain.caillol@enscm.fr (S.C.)

^c QualiSud, Université de Montpellier, Avignon Université, CIRAD, Institut Agro, IRD, Université de la Réunion, Montpellier France

eric.rondet@umontpellier.fr (E.R.)

^d Arthrocart Biotech, Marseille, France

michel.assor@arthrocart.com (M.A.)

^e Department of Pharmacy, Nîmes University Hospital, 30900, Nîmes, France

*Corresponding authors: E-mail address: benjamin.nottelet@umontpellier.fr (B.N.)

Supplementary material

Fig. S1. 2D heteronuclear spectrum ¹ H- ¹ H g-edited COSY (600 MHz, CdCl ₃) of PCL-LDI.	3
Fig. S2. 2D heteronuclear spectra ¹³ C- ¹ H g-edited HSQC (600 MHz, CdCl ₃) of the prepolymer PCL-LDI.	4
Fig. S3. Thermograms of PLGA-diol, PCL-LDI and the resulting PEU.....	4
Fig. S4. Typical pore size distribution of a PEU scaffold.	5
Fig. S5. Typical stress/strain compression curve of HDI-based PEU scaffold.....	5
Fig. S6. Calibration curve of the hydroxyproline content. Linear fit: $y=0.1472x$, $r^2=0.9996$	6
Fig. S7. Calibration curve of the Col-FITC content. Linear fit: $y=2.2022x$, $r^2=0.9985$	7
Fig. S8. Representative μ CT images of A) PEU scaffold, B) PEU-Col scaffold, C) PEU-Col scaffold with iohexol. Red line corresponds to the cross section showed in Fig 4. Scale bar corresponds to 2 mm.	7
Fig. S9. L929 proliferation on the different scaffolds after 2, 4, 9 and 14 days. The fibroblasts were seeded on TCPS (n=6), Actifit® gold standard (n=4), PEU scaffold (n=6) and PEU-Col scaffold (n=6), and cultured for 14 days. * Indicates a significant difference between groups (* $p<0.05$).	8
Fig. S10. Evaluation of scaffolds cell proliferation: L929 fibroblasts morphology observed under microscope	9
Fig. S11. pH evolution of the PBS degradation solutions containing Gold standard and PEU scaffolds samples. The solution was refreshed when pH decreased by 5 %.	10
Fig. S12. Chromatogram of gold standard Actifit® (SEC MALS-THF).	10

Table S1. IEW and HEW of the precursors of interest.....	2
Table S2. dn/dc values obtained for the polymers.....	2
Table S3. Raw data of the absorbance values and hydroxyproline extracted from the calibration curve.....	6

Table S1. IEW and HEW of the precursors of interest.

LDI	PCL-LDI	PCL-diol	PLGA-diol
IEW= 116.2 ± 0.4 g/eq	IEW= $1252 \pm$ g/eq	HEW= $1\ 112 \pm 16$ g/eq	HEW= 936 ± 12 g/eq

Table S2. dn/dc values obtained for the polymers.

	PLGA 50	PCL-LDI	PEU
dn/dc (ml/g)	0.044 ± 0.0026	0.079 ± 0.0025	0.0688 ± 0.0043
R ²	0.9894	0.9957	0.9957

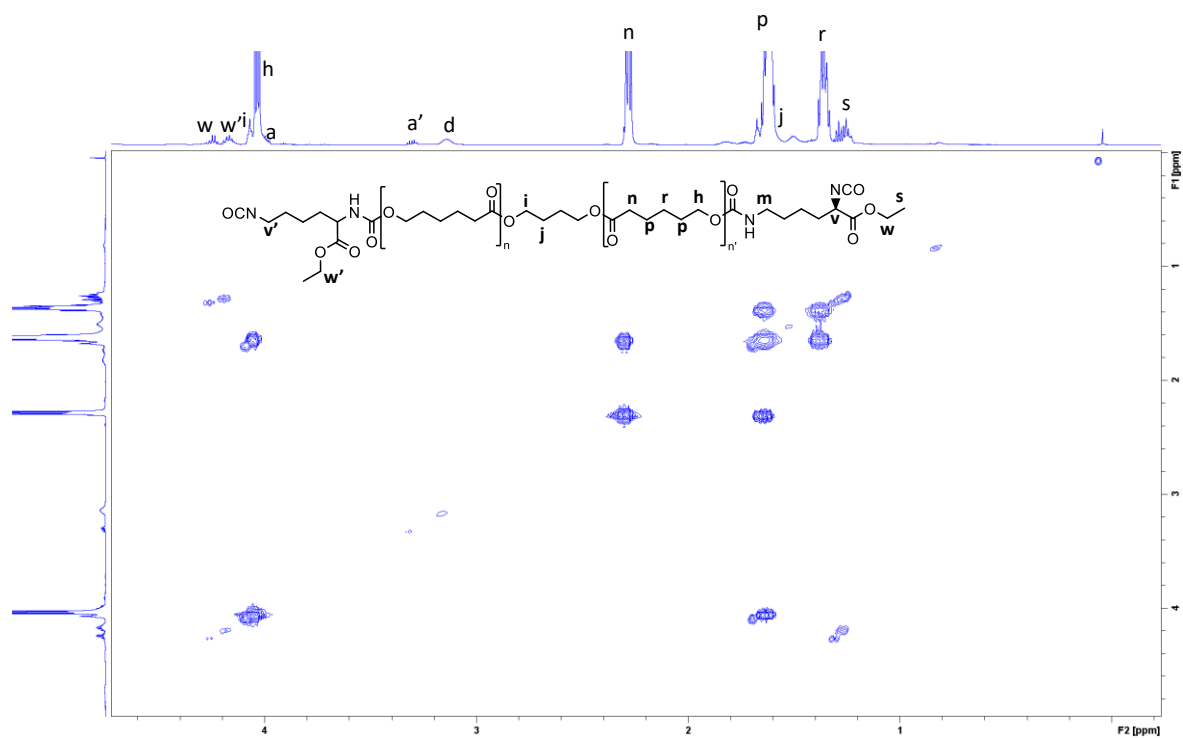


Fig. S1. 2D heteronuclear spectrum ¹H-¹H g-edited COSY (600 MHz, CdCl₃) of PCL-LDI.

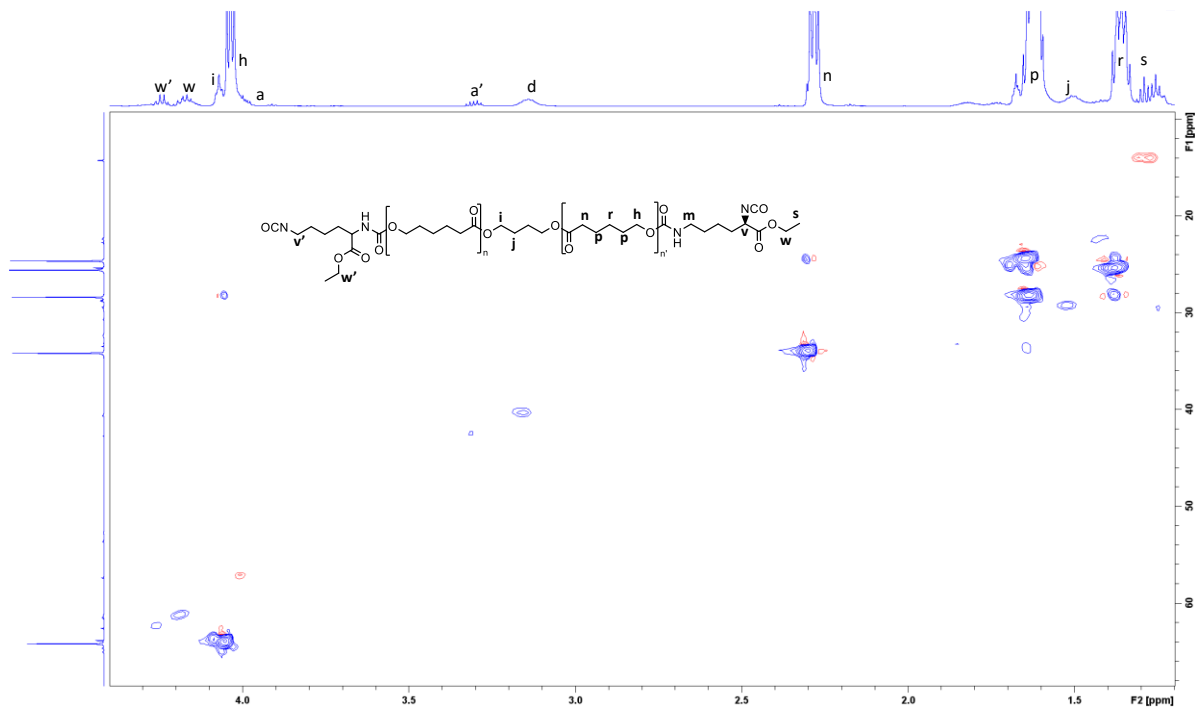


Fig. S2. 2D heteronuclear spectra ^{13}C - ^1H g-edited HSQC (600 MHz, CdCl_3) of the prepolymer PCL-LDI.

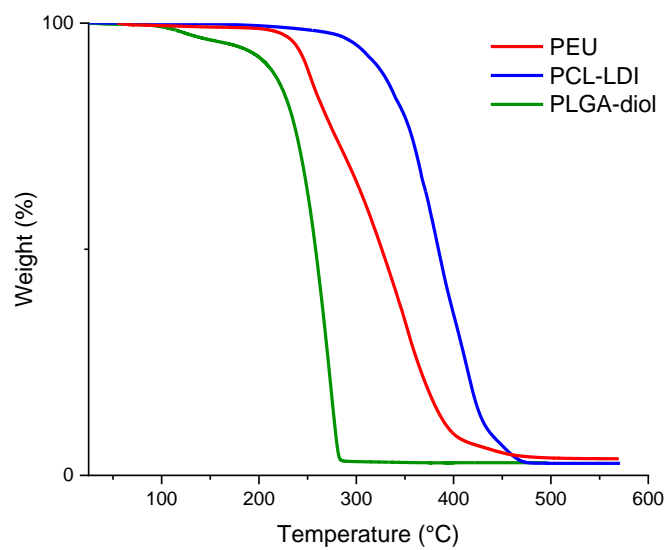


Fig. S3. Thermograms of PLGA-diol, PCL-LDI and the resulting PEU.

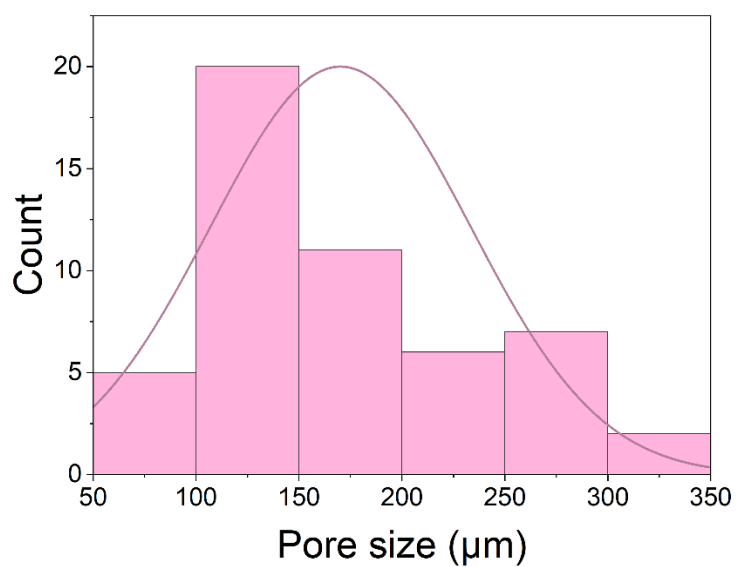


Fig. S4. Typical pore size distribution of a PEU scaffold.

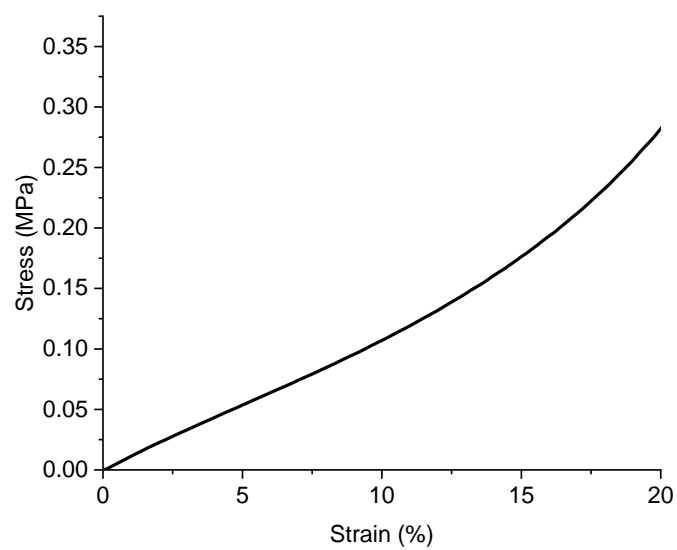


Fig. S5. Typical stress/strain compression curve of HDI-based PEU scaffold.

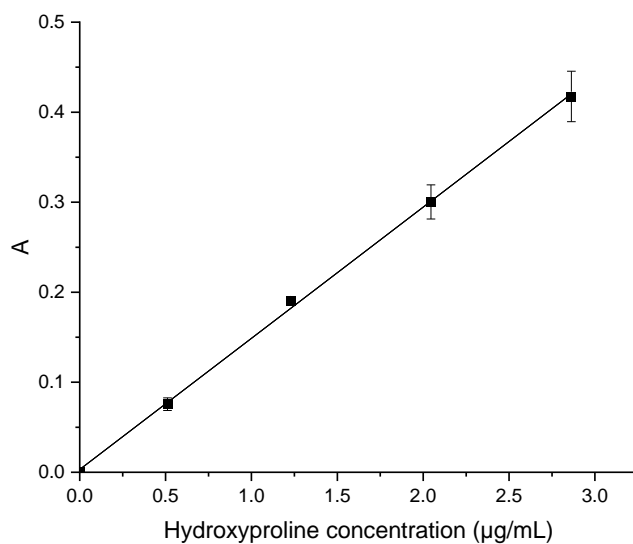


Fig. S6. Calibration curve of the hydroxyproline content. Linear fit: $y=0.1472x$, $r^2=0.9996$.

Table S3. Raw data of the absorbance values and hydroxyproline extracted from the calibration curve.

Sample dilution	Absorbance	Hydroxyproline concentration (µg/mL)	Hydroxyproline mass (µg)	Collagen mass (µg)	Average collagen mass (mg)
1/1	1.422	12.5	31.2	237.4	0.262 ± 0.026
1/2	0.737	6.48	32.4	246.2	
1/10	0.154	1.35	33.8	256.8	

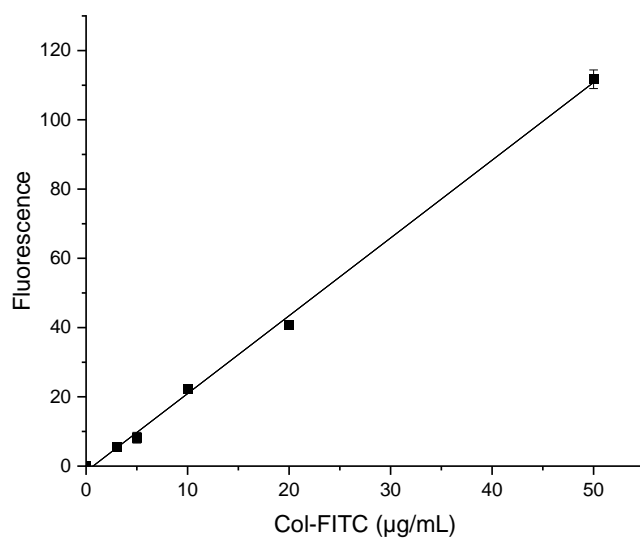


Fig. S7. Calibration curve of the Col-FITC content. Linear fit: $y=2.2022x$, $r^2=0.9985$.

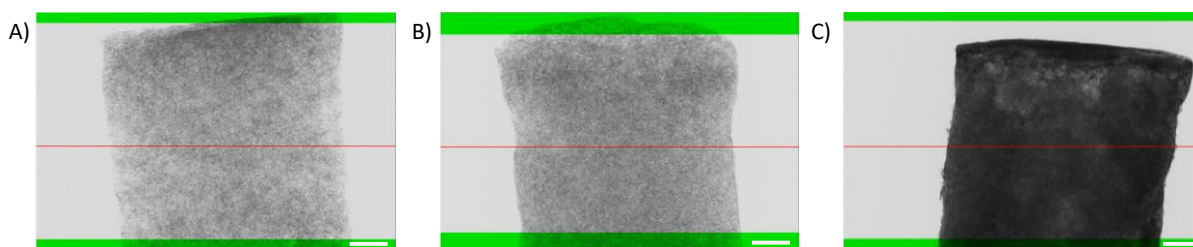


Fig. S8. Representative μ CT images of A) PEU scaffold, B) PEU-Col scaffold, C) PEU-Col scaffold with iohexol. Red line corresponds to the cross section showed in Fig 4. Scale bar corresponds to 2 mm.

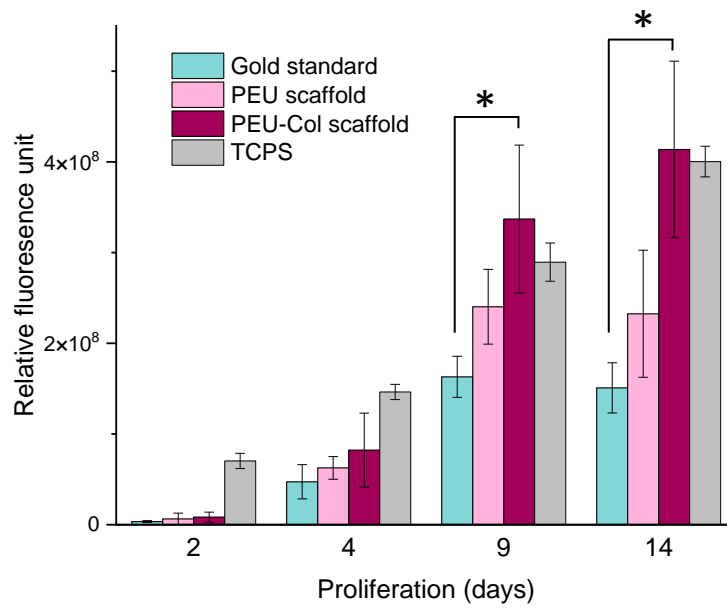


Fig. S9. L929 proliferation on the different scaffolds after 2, 4, 9 and 14 days. The fibroblasts were seeded on TCPS (n=6), Actifit® gold standard (n=4), PEU scaffold (n=6) and PEU-Col scaffold (n=6), and cultured for 14 days. * Indicates a significant difference between groups (*p<0.05).

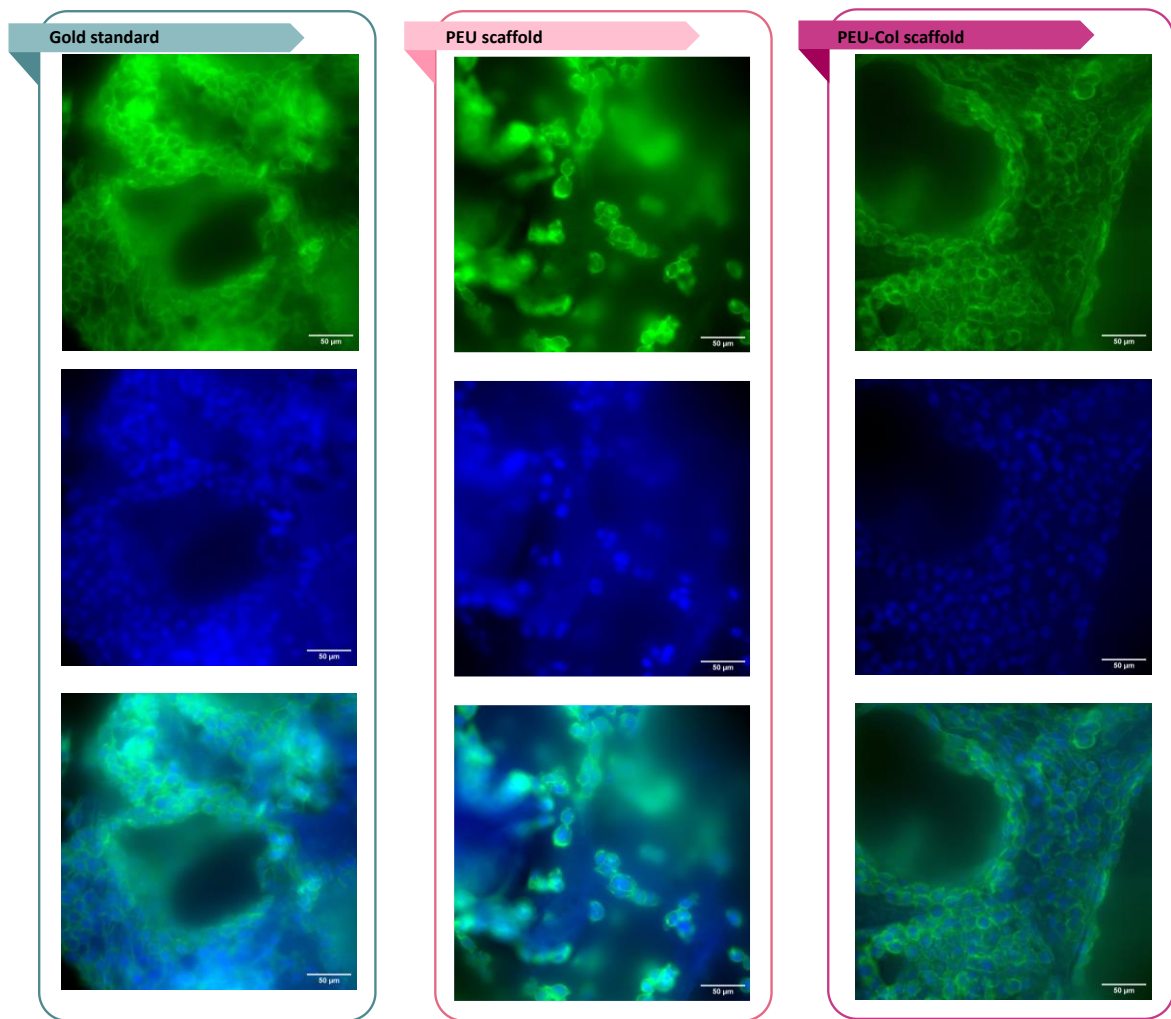


Fig. S10. Visualization of L929 fibroblasts on scaffolds. L929 fibroblasts morphology was observed using phalloidin iFluor 488 (F-actin stained in green) and Hoechst (nucleus in blue) with a Leica Thunder microscope. Scale bars correspond to 50 μm.

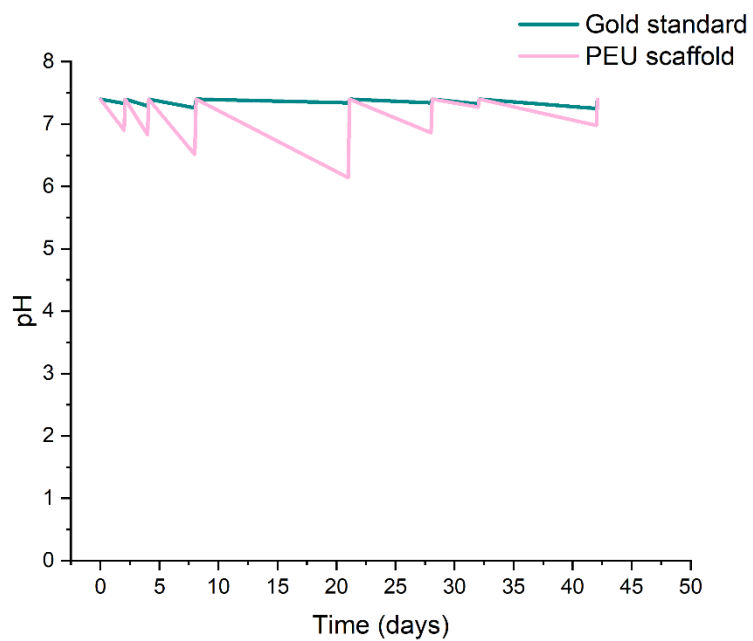


Fig. S11. pH evolution of the PBS degradation solutions containing Gold standard and PEU scaffolds samples. The solution was refreshed when pH decreased by 5 %.

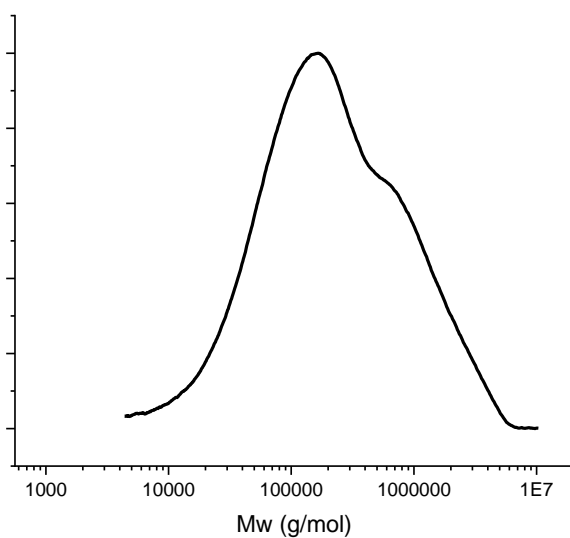


Fig. S12. Chromatogram of gold standard Actifit® (SEC MALS-THF).