

Glucosamine grafting on poly(ϵ -caprolactone): a novel glycosylated polyester as substrate for tissue engineering.

Laura Russo,^a Antonio Gloria,^b Teresa Russo,^b Ugo D'Amora,^b Francesca Taraballi,^a Roberto De Santis,^b Luigi Ambrosio,^b Francesco Nicotra,^a Laura Cipolla*^a

Supplementary Information

The carbon 1s (C 1s) emission spectrum was deconvoluted into four peaks corresponding to increasingly electronegative chemical environments. These are (i) C-C and C-H at 285 eV, (ii) C-O at 286.5 eV, (iii) O-C-O and C=O at around 288 eV (iv) O-C=O at 289.5 eV (Fig. S1, Supplementary Information). Subtle indicators for the presence of bonded glucosamine are provided in the C1s spectrum of glycosylated PCL (Fig. S1, Supplementary Information), where a slight increment of the C-O contributions can be observed.

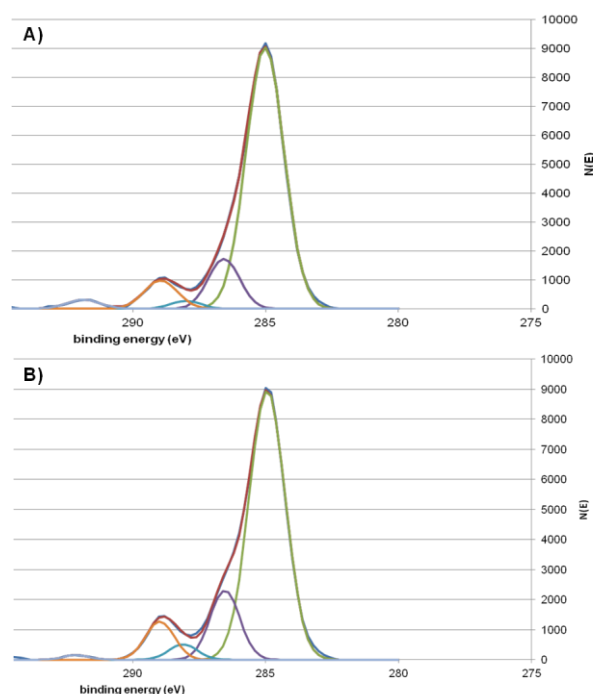


Figure S1. XPS analysis and curve fitting for the (C 1s) emission spectra: (a) plain PCL; (b) glycosylated PCL.

Human Mesenchymal Stem Cells (hMSCs, $5 \cdot 10^4$ cells/sample) were seeded on unmodified and glucosamine-bound PCL substrates and grown in DMEM w/o FBS. Then, Alamar BlueTM (AbD Serotec Ltd, UK) assay (Fig. 2S) was performed on the cell constructs in order to assess cell viability. Briefly, this method is based on a redox reaction that occurs in the mitochondria of the cells and the colored product is transported out of the cell and can be measured spectrophotometrically. The optical density was measured using a spectrophotometer (Sunrise; Tecan, Männedorf, Zurich, Switzerland) at wavelengths of 570 and 595 nm. According to the manufacturer's protocol, the number of viable cells correlates with the magnitude of dye reduction and is expressed as a percentage of Alamar BlueTM reduction. Each experiment was performed at least three times in triplicate. Results obtained from Alamar BlueTM are represented in Fig. S2.

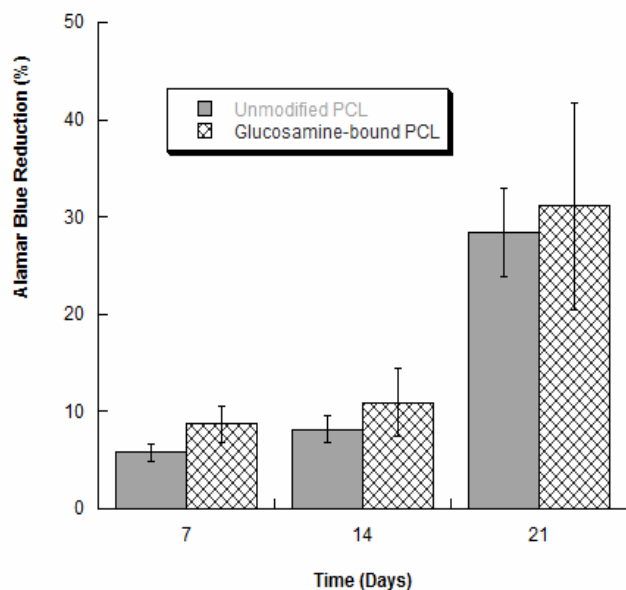


Figure S2. Alamar Blue™ assay performed on cell-scaffold constructs: results (mean value \pm standard deviation) reported at 7, 14 and 21 days.

Instrumentation

ATR-FTIR.

ATR-IR absorption spectra were recorded at RT in the range 800–3500 cm^{-1} with a micro-FTIR Nicolet iN10, equipped with a micro-ATR germanium tip, under nitrogen flux, with a spectral resolution of 1 cm^{-1} and 256 accumulations. The measurements have been done 3 times in different regions of PCL sample in order to verify the homogeneity of the functionalization. In the same conditions a bare PCL sample has been analyzed. After baseline subtraction, spectra were normalized to unity with respect to the peak centered around 1735 cm^{-1} , which is typical of the stretching vibration of free bonded carbonyl.

XPS

X-ray Photoelectron spectroscopy analysis was performed with a Perkin Elmer PHI 5400 ESCA System (anodic source Mg, 10kV power 200 W). Software AugerScan Ver. 3). The instrument shift of b.e. has been corrected using 285 eV internal standard for C-C C1s component.

All chemicals were purchased from Sigma-Aldrich and used without further purification.