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ARTICLE ELECTRONICAL SUPPLEMENTARY INFORMATION

Towards 4th generation biomaterials: a covalent hybrid polymer-ormoglass architecture

N. Sachot,[ab] M.A. Mateos-Timoneda,[bae] J. A. Planell,[ab] A.H. Velders[c] M. Lewandowska,[d] E. Engel[abe] and O.Castaño.*[abf]

*Corresponding author: ocastano@ibecbarcelona.eu

Zeta potential measurements

Zeta Potential of the fibrous samples after the different surface treatments was determined by the streaming current approach. The pH of the electrolyte was titrated from the basic region (pH~9) to the acidic one (pH~2.5) by adding HCl 0.1M using the device pump. The electrolyte was forced to pass between two thin layers of fibers using a pressure program of maximum 300 mbar. The curves obtained for each sample are presented in **figure S1**. The isoelectric points and Zeta potentials at pH=7 reported in the article (**Table 1**) were extracted from this graphic. Changes in the zeta potential are observed after each surface modification. This suggests that the different surface functionalizations were efficiently achieved.

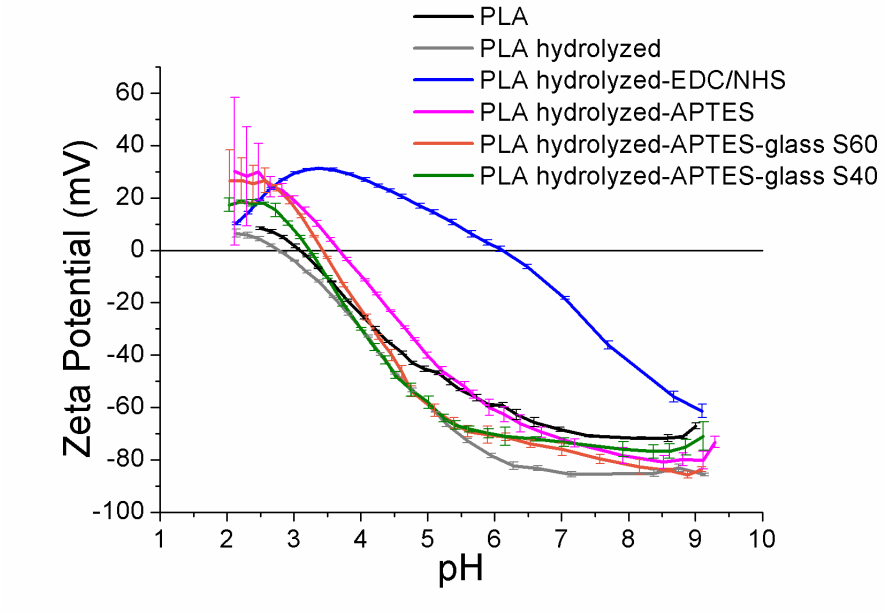

Figure S1. pH dependence of the zeta potential of the fibrous layer after the different surface treatments applied to perform the coating.

EDS spectra

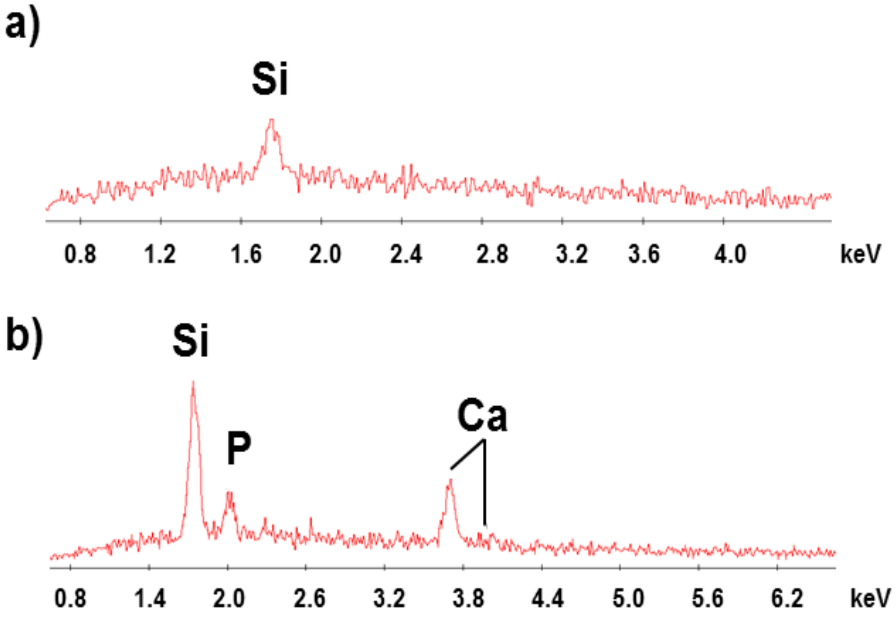

Figure S2. EDS spectra related to (a) APTES functionalized fibers and (b) coated fibers.

FTIR spectra of the coated fibers acquired by ATR method

FTIR spectra obtained for the PLA fibers, hydrolyzed and APTES functionalized fibers, and coated fibers (S40 and S60).

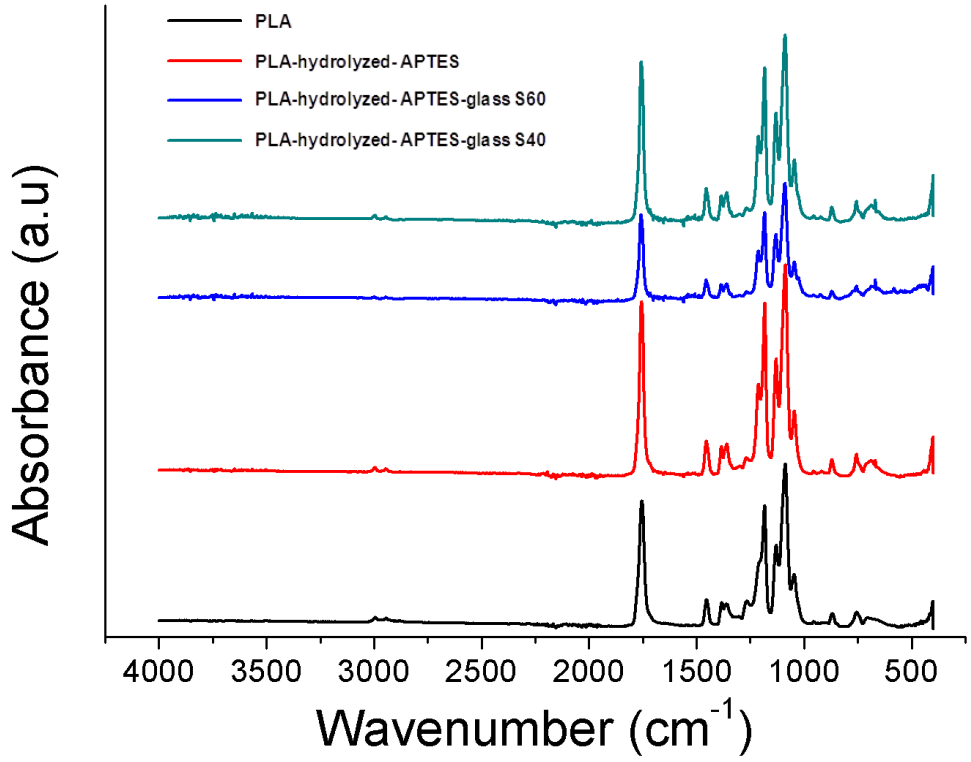

Figure S3. Full FTIR spectra obtained for the PLA fibers, hydrolyzed and APTES functionalized fibers, and coated fibers (S40 and S60).

Dynamic light scattering

Dynamic Light Scattering (DLS) measurements were performed to determine the size of the ormoglass particles of both compositions. Ormoglass particles were prepared as explained in the article; by hydrolyzing during 2 min 30 the ormoglass precursor solutions (mix) with different Si:H_2O water ratio (1:0.5, 1:1, 1:2, 1:3 for the S60 ormoglass and 1:1, 1:2, 1:3, 1:4, 1:5 for the S40 one). After this time, 1mL of absolute ethanol was added to 0.5 mL of particle suspension and vigorously shaken. This dilution was done to minimize further hydrolysis of the solution that could occur during the centrifuge that followed. Centrifugation (4 minutes at 20°C and 4000 rpm) was done to collect the particles. The supernatant was carefully removed and 1.5mL of absolute ethanol was added to the deposit. Particles were homogeneously re-suspended and centrifuged again. This step was performed twice to remove the excess of ormoglass precursor mix and to have finally the particles in suspension in pure absolute ethanol. The ormoglass particles suspension obtained was sonicated for 1 minute before starting the measurements and 1mL of that solution was placed in the measurement "cuvette" of the device. Measurements were conducted using a Malvern Zetasizer Nano ZS device (laser wavelengths of 633 nm and detection angle of 173°) and were run 3 times with 2 minutes of intervals for each types of suspension. DLS results were analyzed using Malvern DTS software v5.1. Results demonstrated that the S60 particles were in all the cases bigger than the S40 ones (**figure S4**). It also showed that the diameter of the S60 particles is significantly influenced by the level of hydrolysis, whereas the diameter of the S40 particles remained quite stable independently of the hydrolysis ratio considered. These observations explain the difference in roughness and coating thickness between the coated fibers with the both ormoglass compositions.

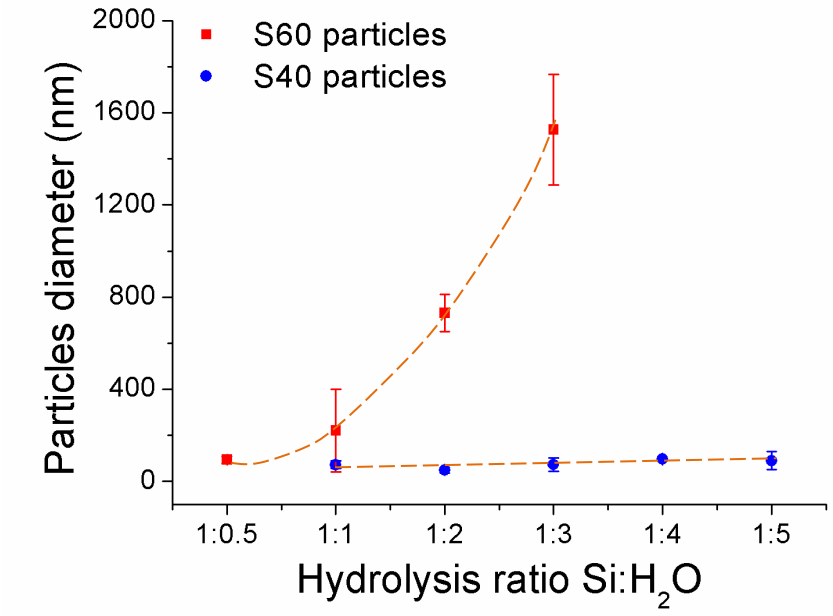

Figure S4. Comparison between the particles size of S40 and S60 particles prepared with different hydrolysis ratios.

FESEM observations of the remaining inorganic phase after thermal degradation of the coated fibers

The remaining inorganic shell of the coated fibers obtained after thermal degradation of the organic parts (PLA and organic fragments of the ormoglass) was analyzed under FESEM to assess whether the coating was made of a mono or multiple layer of ormoglass particles (**figure S5**). Pictures were also used to evaluate the thickness of the coating. It was however kept in mind that this measured thickness was a reduced value of the real thickness because organic fragments, initially contained in the ormoglass (organic/inorganic glass obtained by the partial hydrolysis of alkoxide precursors), decomposed during the thermal treatment. As a consequence, a decrease of the shell thickness surely occurred. Images showed that the coating of the S60 fibers was made of monolayer of particles. For the S40 fibers, images revealed the collapsing and fragility of the remaining inorganic compound (**figure S5**).

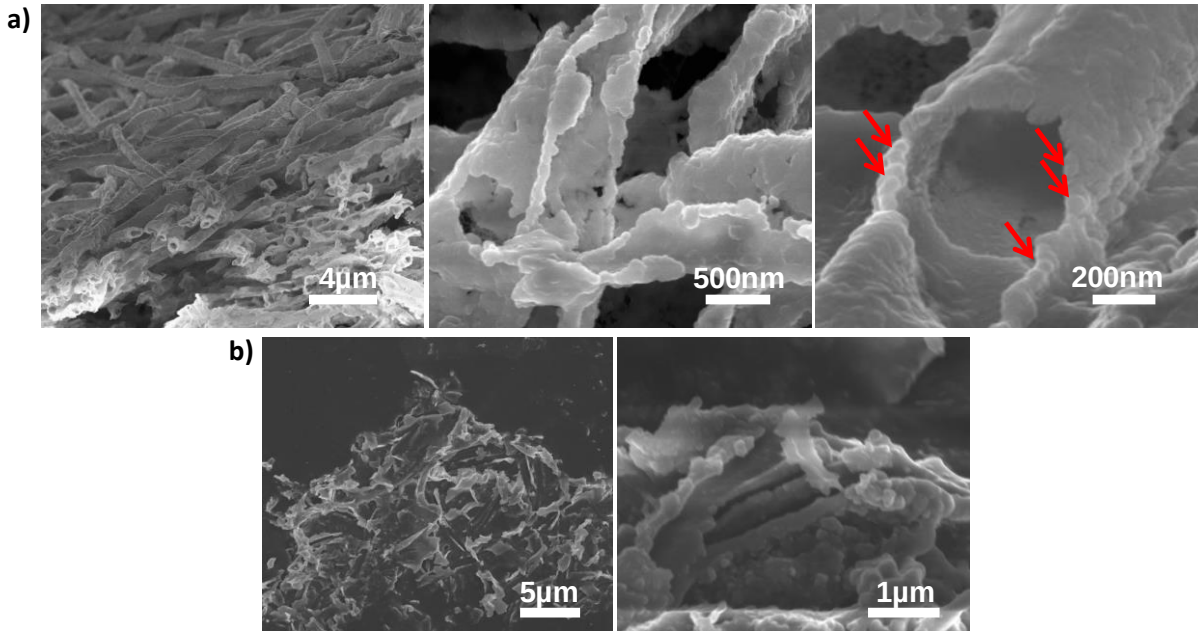

Figure S5. Morphology of (a) PLA fibers coated with the S60 ormoglass composition showing the particles monolayer (arrows) and the thickness of the coating after thermal treatment, and morphology of (b) PLA fibers coated with the S40 ormoglass composition showing the fragility and collapsing of the sample after thermal treatment.

Thermogravimetric analysis coupled Fourier Transform Infrared spectroscopy

FTIR was coupled with TGA analysis in order to determine the nature of the compounds that degraded during the heating temperature program applied to the fibers coated with the S60 ormoglass. This method enables the acquisition of the FTIR spectra of the gaseous products that vaporized at different temperatures to be used for compound identification. The spectra related to the decomposition of the three compounds seen with TGA are presented in **figure S6**. Based on spectral database of the literature,[1,2] the peak at 77°C was attributed to the presence of triethoxysilane compounds. This is due to the use of TEOS for the preparation of the ormoglass and possible residual remaining molecules not incorporated in the ormoglass network. Peaks at 270°C and 360°C were both assigned to the decomposition of different PLA fragments (lactate and other compounds associated to the degradation of PLA: carbon dioxide, carbon monoxide, acetaldehyde…).[3]

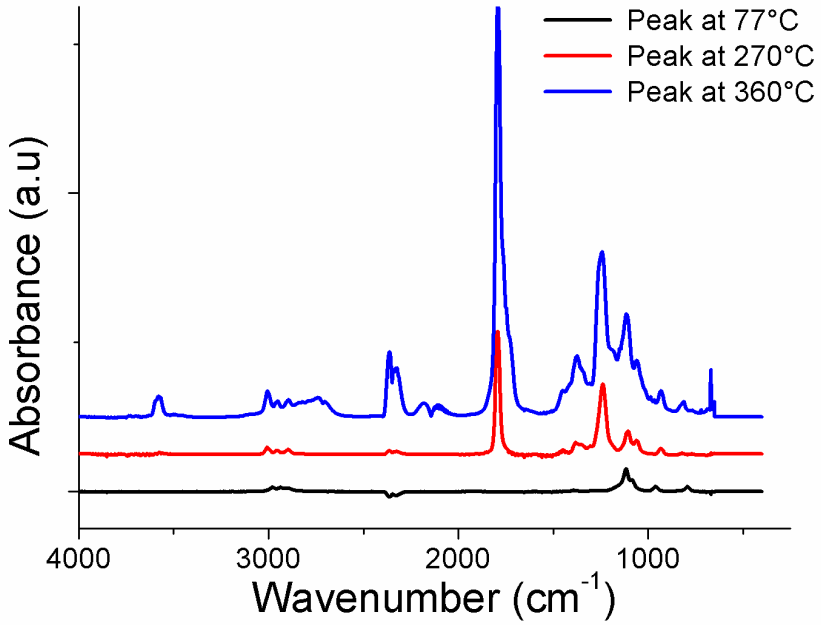

Figure S6. FTIR spectra of compounds vaporizing during the thermal degradation of PLA fibers coated with glass S60 at 77°C, 270°C and 360°C.

Gas chromatography assay

Although it was evident for the fibers coated with the S60 ormoglass that they possessed residual TEOS molecules, TGA did not allow clear conclusion for the ones coated with the S40 ormoglass; probably due to the small amount of ormoglass linked to these fibers. Gas chromatography was therefore used to see if silane molecules could be however detected by using another technique, hypothesizing that they were, in fact, present in the ormoglass particles. Sample of 1x1 cm^2 was incubated for 48h in Millipore water and the supernatant was analyzed using a Trace GC Ultra device (Thermo Scientific ITQ 900). The results shown in **figure S7** confirmed that residual TEOS molecules were also found in the S40 coated fibers. Triethoxypropylsilane molecules from APTES were also detected but in a smaller amount than TEOS.

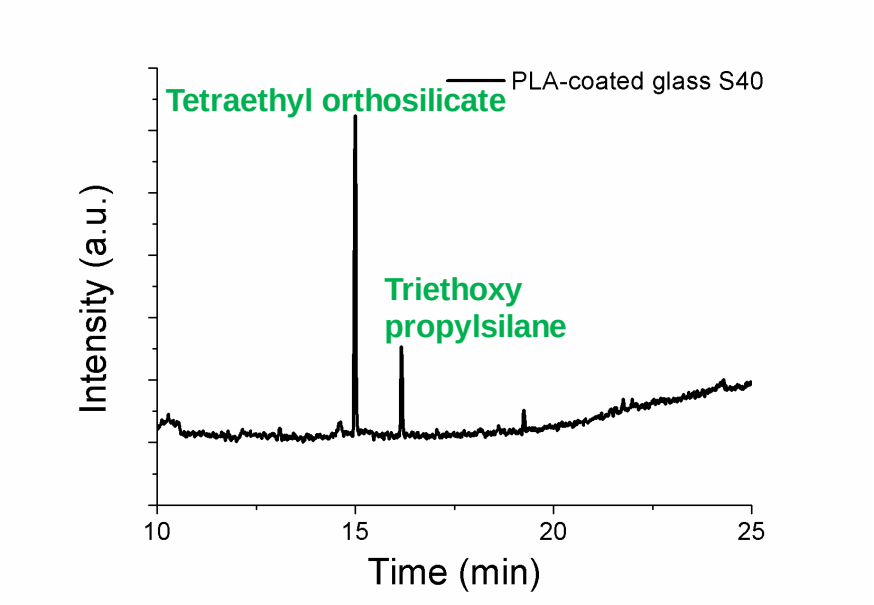

Figure S7. Gas chromatography pattern of fibers coated with S40 ormoglass, showing the presence of TEOS.

Differential scanning calorimetry assays

Thermal properties of the organic phase of the coated fibers were assessed using differential scanning calorimetry. Data reported in Table 4 of the article to compare the degree of crystallinity of pristine PLA and the coated fibers were calculated using the curves plotted in **figure S8**. Increase of the crystallinity and changes in the different characteristic transition temperatures can be noticed.

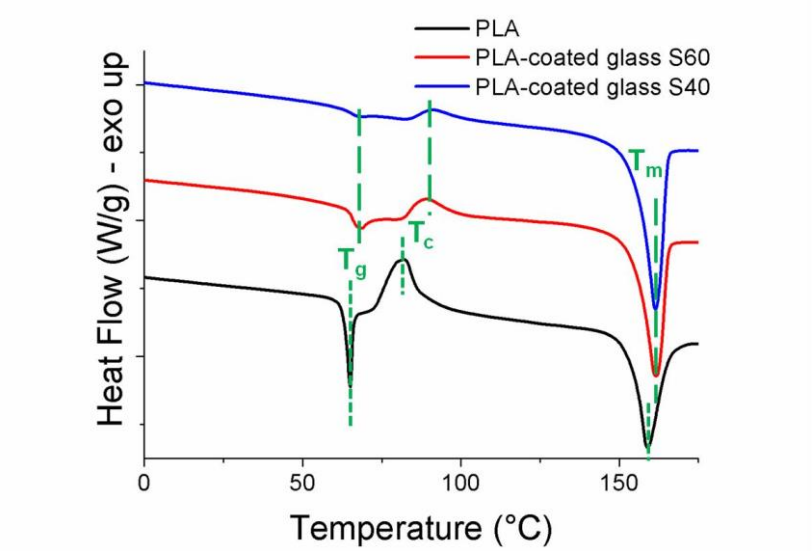

Figure S8. DSC thermograms of PLA and hybrid coated fibers.

Fibers crystallinity

X-Ray assays

X-Ray diffraction method was used to confirm the increase of crystallinity of the PLA fibers after their coating. The assay was conducted using a PANalytical X'Pert PRO MPD diffractometer and measuring from 2 to 40° 2θ. According to DSC quantification, pristine PLA fibers exhibited a low crystallinity level that was not detected by X-ray diffraction (**figure S9**). On the contrary, for the coated fibers, a peak characteristic of the polymeric phase appeared. X-ray diffraction assay confirmed thus the DSC measurements associated to the coated fibers and the increase of crystallinity after coating. It demonstrates, moreover, that the ormoglass particles prepared by the sol-gel method were indeed amorphous. Finally, it showed that the processing of the PLA influences its structural organization: as pellets, PLA was a little bit crystalline, whereas after being electrospun it was almost completely amorphous. One explanation to that phenomenon can be that the solidification of the liquid jet during the electrospinning process is too fast to enable an eventual phase arrangement.

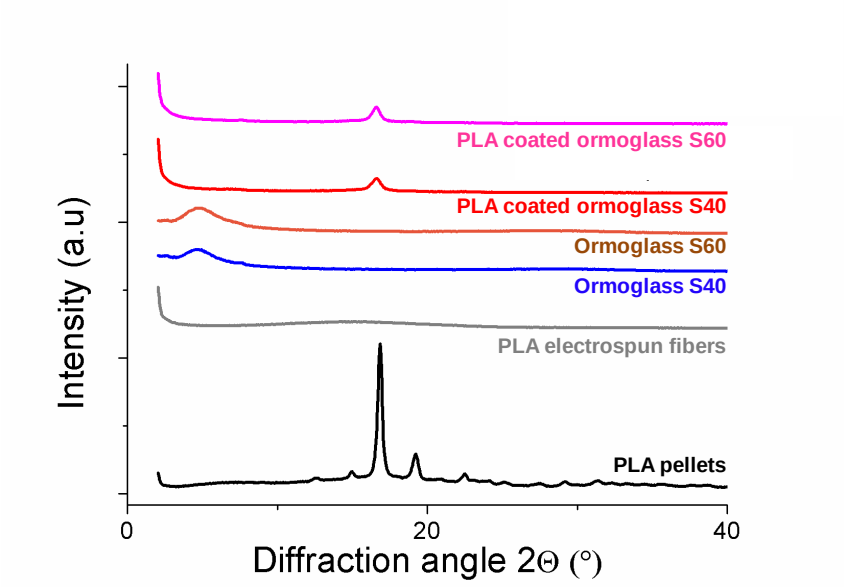

Figure S9. X-ray diffraction spectra of PLA pellets (pellets dissolved to obtain the polymeric solution for electrospinning – raw material), PLA electrospun fibers, S40 and S60 ormoglasses, and PLA fibers coated with both ormoglass compositions.

DSC assays

It was initially suggested that the increase of crystallinity of the fibers after the coating resulted from the first treatment applied to the fibers: the hydrolysis. As hydrolysis induces the shortening of polymer chains, it was hypothesized that the chains could have rearranged and promoted the crystallization of some areas. To verify this hypothesis, PLA (raw fibers) and hydrolyzed-PLA fibers were both analyzed by DSC (Mettler Toledo DSC 822e calorimeter, TA STARe evaluation software v12.10). Samples were heated up to 200°C (10°C·min^{-1}). The percentages of crystallinity revealed that no significant differences exist between both fiber types (**figure S10**). This demonstrated that the hydrolysis did not cause the increase of crystallinity. For this reason, it was believed that the coating with the ormoglass was the explanation.

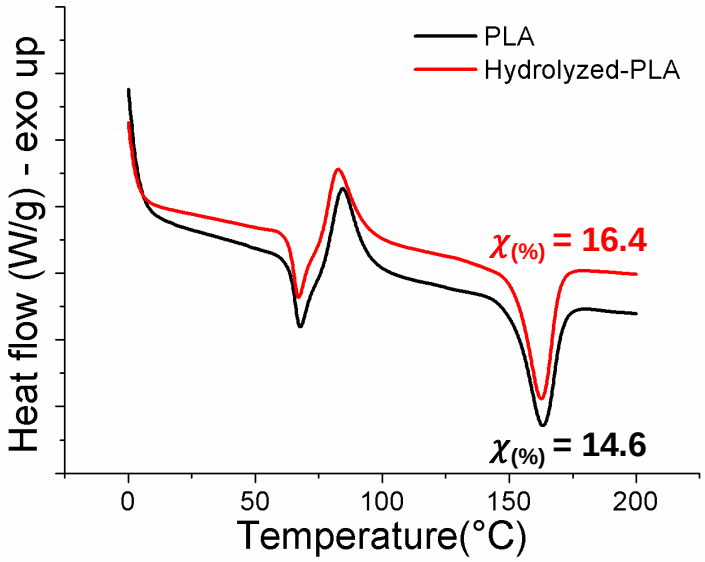

Figure S10. DSC thermograms of PLA and hydrolyzed-PLA fibers (χ: percentage of crystallinity).

Isolation and culture of endothelial progenitor cells and mesenchymal stem cells from rat bone marrow

Lewis rats (Charles-River) were sacrificed and femora and tibiae were surgically removed. Bone marrow was obtained by gently flushing the inside of the bones with medium 199 (Sigma) supplemented with 20% FBS, 1% Penicillin / Streptomycin, 1% L-Glutamin (Invitrogen), 1% sodium pyruvate (Invitrogen) and 22.5 $\mu g \cdot ml^{-1}$ heparin (Sigma). Then, the whole cell fraction was moved to 1 $\mu g \cdot ml^{-1}$ fibronectin pre-coated 6-well plates (Sigma). After 1 day, the supernatant was then recovered and transferred to a new well. 24 hours later the supernatant was again replated in another well and medium was replaced by M199 supplemented with 20% FBS, 1% 1% Penicillin / Streptomycin, 1% L-Glucose (Invitrogen), 1% sodium pyruvate, 22.5 $\mu g \cdot ml^{-1}$ heparin, 1 $\mu g \cdot ml^{-1}$ ascorbate, 20 $ng \cdot ml^{-1}$ IGF, 5 $ng \cdot ml^{-1}$ EGF (Sigma), 20 $ng \cdot ml^{-1}$ VEGF and 10 $ng \cdot ml^{-1}$ bFGF (Peprotech). Cells reached confluence after 10-12 days and showed typical cobblestone morphology, occasionally forming long tube-like structures. They were positive for EPC markers vWF, CD31, CD34, VEGFR-2, UAE-1 and were able to form tube-like structures in matrigel (data not shown). Mesenchymal stem cells were obtained by a similar procedure: after flushing the bone marrow with the medium described above the whole cell fraction was plated in plastic Petri dishes and allowed to stay for 1 day, after which the medium containing the non-adherent cell fraction was removed and changed to ADMEM (Sigma) supplemented with 15% FBS, 1% Penicillin / Streptomycin, 1% L-Glucose (Invitrogen). After 2-3 days it was possible to observe quickly proliferating colonies of MSCs. Cells were trypsinized at subconfluence and passaged 1:3. Cells from passages 3 to 6 were used.

References:

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