Electronic Supplementary Material (ESI) for Biomaterials Science.

Long-term in vivo performances of polylactide / iron oxide nanoparticles core-shell fibrous nanocomposites as MRI-visible magneto-scaffolds

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Synthetic procedures

Preparation of super magnetic iron oxide nanoparticles (SPIONs). Oleic acid-functionalized SPIONs were synthesized by method similar to those reported in the literature.[1] Briefly, 0.18 g of FeOOH fine powder, 3.2 g of oleic acid and 5.00 g of *n*-docosane were combined and evacuated for 30 min. Then, the mixture was heated under argon at 340 °C for 1.5 h. The reaction mixture was allowed to cool to approximately 50 °C and diluted with 15 mL of pentane. Furthermore, the nanoparticles were purified and isolated after successive dispersion-centrifugation cycles using diethyl ether: ethanol mixtures (2:1 and 1:1 ratios) at 20,000 rpm for 10 min, with removal of the supernatant containing organic components. The purification was repeated several times until the supernatant solution became colorless. Finally, the SPIONs were redispersed in cyclohexane (20 mL) in the presence of additional oleylamine (200 µL), and freshly used in further experiments. TEM analyses were performed on these nanoparticles, showing that their average diameter was ~18 nm.

Propargylation of PLA fibers. In a flamed-dried 3-Neck Lab reactor with lid and steel clamp, 100 mL of dry Et_2O was introduced under argon atmosphere and the medium was cooled to −20 °C. Then, the PLA fibrous mesh (8 cm^2) placed in a cylindrical glass holder to prevent contact with magnetic stirrer was immerged into the solvent. The solution was saturated with argon for 15 min, before LDA (2M solution, 1 mL, 2 mmol) was added. After 15 min, a propargyl bromide solution (80 wt% in toluene, 1 mL, 9 mmol) saturated with argon was injected, and the mixture was stirred for an additional 15 min at −50 °C. The reaction was quenched with water and the rack was washed several times in a cold diethyl ether bath for 30 min, before drying under argon atmosphere.

Preparation of PLA@SPIONs hybrids

This two step-procedure was reported in our previous work, which can be refered to for illustrative schemes.[2]

Step 1: Phosphonic acid-functionalized PLA fibers through thiol-yne photo-addition. In a flame-dried 3-neck Lab reactor with lid and steel clamp protected from light and placed under argon atmosphere, 12-mercaptododecylphosphonic acid (0.38 mg, 1.34 µmol) and DMPA (0.34 mg, 1.34 µmol) were added to 80 mL of a cyclohexane/ acetone mixture (3:1 ratio). The solution was saturated with argon prior to immersion of a Teflon rack holding 10 glass plates covered by propargyl functionalized PLA fibers. Then UV irradiation (100 $mW.cm^{-2}$) was carried out for 10 min under gentle stirring. Following the thiol-yne photo-addition, PLA fibers were washed several times using the solvent mixture and dried under argon atmosphere.

Step 2: SPION-functionalized PLA fibers through free ligands exchange procedure. A 100 mL cyclohexane/ acetone mixture (3:1 ratio) was introduced into a flame-dried 3-neck Lab reactor with lid and steel clamp under argon atmosphere. Then, a Teflon rack holding 10 phosphonic acid-functionalized PLA fibers was immersed in the solution before addition of an excess of freshly prepared oleic acid-functionalized SPIONs (2 mg) dispersed in cyclohexane solution with a concentration of 1 $mg.mL^{-1}$ and sonicated in an ultrasonic bath for 30 min. The reaction was protected from light and stirred for 24h at room temperature under argon. To remove the free SPIONs not anchored to the surface, the SPION-functionalized PLA fibers were cleaned by successive washings in a cyclohexane/acetone mixture, and dried under argon. A change in color from white to light brown was clearly observable for the PLA fibers. The desired material was stored in the fridge before performing further characterizations.

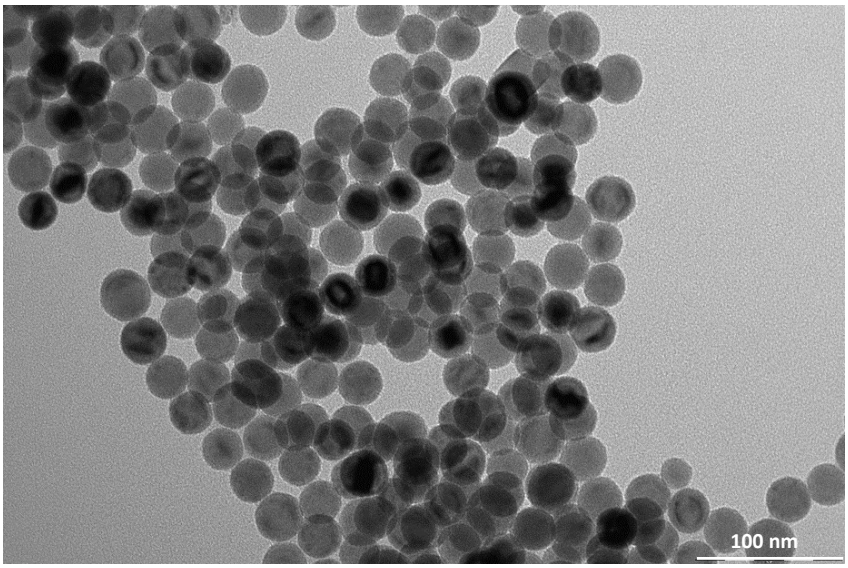

Figure S1. TEM image of oleic acid functionalized SPIONs.

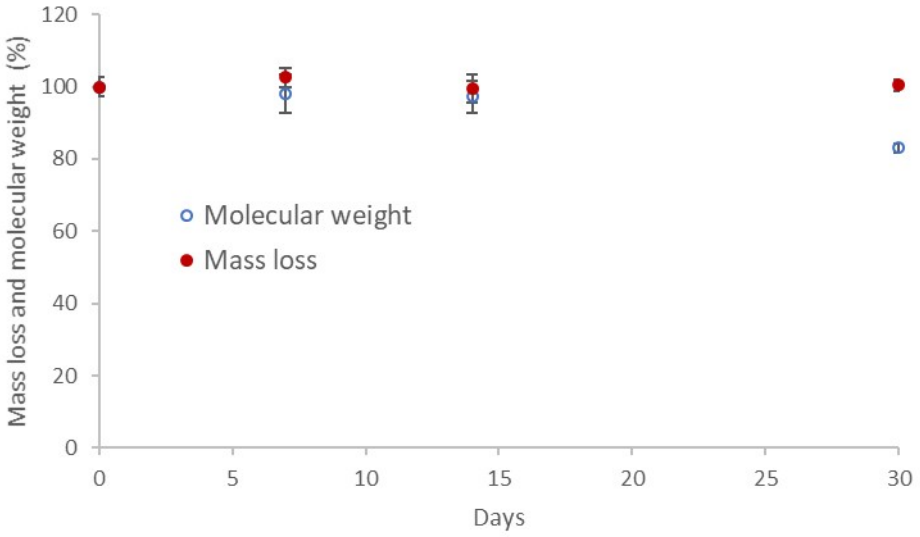

Figure S2. Mass loss and evolution of number average molecular weight (Mn) of PLA@SPIONs samples over 1 month under *in vitro* mimicking physiological conditions (PBS, pH 7.4, 37°C)

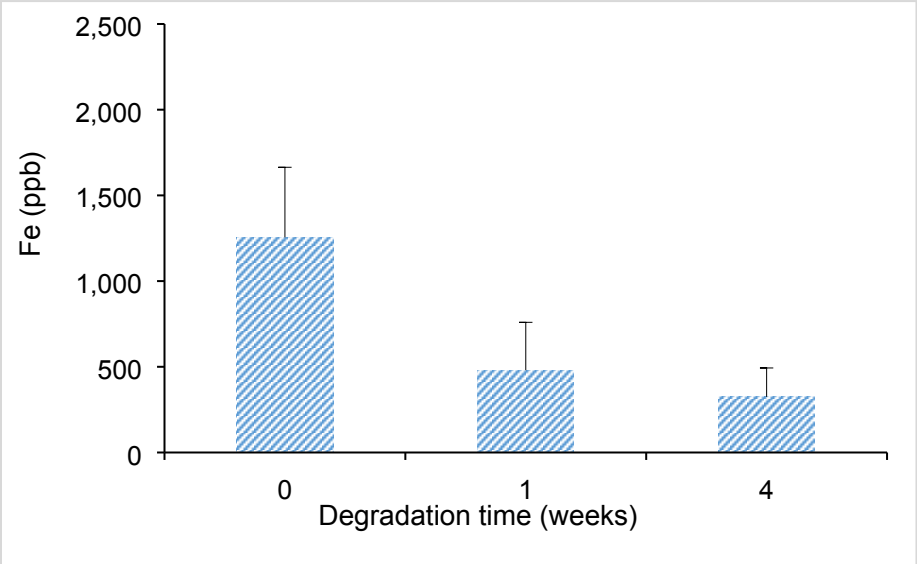

Figure S3. ICP-OES follow-up of the residual Fe present on the PLA@SPIONs nanocomposites upon degradation in PBS.

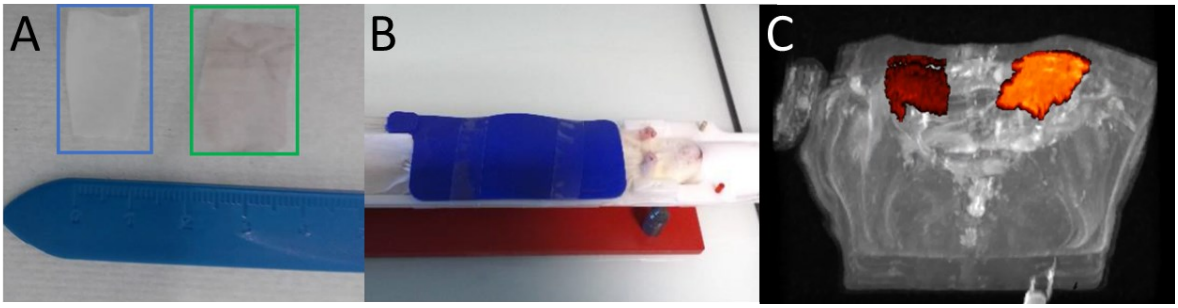

Figure S4. Scaffolds, set up, and 3D-reconstruction of rat abdominal cavity during the i*n vivo* 6-month follow-up of PLA@SPIONs nanocomposites using magnetic resonance imaging. (A) PLA (blue rectangle) and PLA@SPIONs (green rectangle) nanofibrous scaffolds used for implantation (samples were resized to 1 cm^2 before implantation), (B) setup for rat MRI experiments and (C) MRI 3D-reconstruction of rat abdominal cavity at 2 weeks with PLA (orange) and PLA@SPIONs (dark red) nanofibrous scaffolds (the coloration corresponds to artificial colors attributed for illustration purposes).

Table S1. Pathologic evaluation at 3 months post-implantation.

Evaluation of quantification 0= absent, 1= slight, 2= mild, 3= moderate, 4= severe	PLA Score	PLA@SPIONs Score
CELL RESPONSE		
Macrophages	3	3
Multinucleated Giant Cells	3	3
With fibers inside	3	3
Lymphocytes diffused	2	2
Lymphocytes clusters	0	0
Neutrophils	0-1	0-1
Eosinophils	1	1
Quantification of mastocytes (q) and distribution (d)	q1d1	q1d1
Blue staining with Perls		
Blue granular cell content intensity and distribution with the Perls staining (i & d intensity & distribution)	i0d0	i1d2 to i3d1
TISSUE RESPONSE		
Local infiltration of lymphocytes from vessels	0	0
Oedema	0	0
Connective Tissue Organization	1	1
Fibrosis	1	1
Fatty infiltration	1	1

Table S2. Pathologic evaluation at 6 months post-implantation.

Evaluation of quantification 0= absent, 1= slight, 2= mild, 3= moderate, 4= severe	PLA Score	PLA@SPIONs Score
CELL RESPONSE		
Quantity of inflammatory cells per HPF REMOVE		
Macrophages	2	2
Quantity of Multinucleated Giant Cells	3	3
With Crystal inside	3	3
Lymphocytes diffused	2-3	2-3
Lymphocytes clusters	1-3	1-3
Neutrophils	0	0
Eosinophils	1	1
Quantification of mastocytes (q) and distribution (d)	q1d1	q1d1
Blue staining with Perls		
Blue granular cell content intensity and distribution with the Perls staining (i & d, intensity & distribution)	i0d0	i1d1 to i3d2
TISSUE RESPONSE		
Local infiltration of lymphocytes from vessels	2	2
Oedema	2	2
Connective Tissue Organization	2-3	2-3
Fibrosis	2-3	2-3
Fatty infiltration	2	2

Movie S1. MRI 3D-reconstruction of rat abdominal cavity 2 weeks after implantation of PLA or PLA@SPIONs scaffolds. Available on the publisher website.

References :

1. W. W. Yu, J. C. Falkner, C. T. Yavuz and V. L. Colvin, *Chem. Commun.* 2004, **20**, 2306−2307.

2. H. Awada, A. Al Samad, D. Laurencin, R. Gilbert, X. Dumail, A. El Jundi, A. Bethry, R. Pomrenke, C. Johnson, L. Lemaire, F. Franconi, G. Félix, J. Larionova, Y. Guari and B. Nottelet, *ACS Applied Materials & Interfaces*, 2019, **11**, 9519–9529