

Electronic Supplementary Information

Experimental details

All reactants were analytical grade. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (Labsynth, Brazil) 0.1 mol/L and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ 0.05 mol/L (Labsynth, Brazil) were vigorously mixed. NH_4OH 25 vol % (Labsynth, Brazil) was added to the system under stirring until the solution achieved pH 9. Nitrogen gas was bubbled directly into the media prior to reaction for removal of oxygen. Then the system was placed in a water bath at $(24 \pm 1)^\circ\text{C}$, in which the sonotrode MS7 with acoustic power density of 130 W/cm^2 coupled to the Ultrasonic processor Hielscher UP100H was immersed. The sonotrode was not inserted inside the reaction flask in order to avoid contamination by Ti particles stemming from the sonotrode. The sonotrode operated during 10 minutes; the temperature inside and outside of the reaction flask remained at $(24 \pm 1)^\circ\text{C}$. After that, the dispersion containing the magnetite particles (MP) was neutralized and the MPs were separated by centrifugation at 3000 rpm during 10 minutes. The MPs were re-dispersed in MilliQ water and again separated. This rinsing process was repeated three times in order to remove the excess of reactants. After that, part of the MP dispersion was freeze-dried for further characterization and the rest was used for the experiments with xanthan films. These particles were coded as MP-US. For comparison, MPs were also synthesized by a conventional method,⁶ where the system is heated at $(75 \pm 1)^\circ\text{C}$ during one hour instead of using the sonotrode. These particles were coded as MP-Heat. The rinsing process was repeated for the MP-Heat particles. One should notice that no stabilizer was added to the dispersions of MP-US or MP-Heat. In both cases, the concentration of magnetites in the dispersion was $(48 \pm 2) \text{ g/L}$.

Xanthan ($M_v \sim 1,0 \cdot 10^6 \text{ g/mol}$, degree of pyruvyl = 0.38, degree of acetyl = 0.41, Kelco, USA) chains were cross-linked in the presence of citric acid, as described elsewhere (Bueno et al., 2013) Such cross-linked xanthan films are $25 \pm 5 \mu\text{m}$ thick, stable in the pH range of 2 to 9, and after swelling in water (under equilibrium conditions) their mass increase up to 27 times its original dried mass. The xanthan films were immersed in the MP dispersions at pH 7 and $(24 \pm 1)^\circ\text{C}$ during 10 seconds. After that, the films were removed and rinsed in MilliQ water for 20 seconds. This process was repeated three times more in order to remove the MPs, which were weakly attached to polymeric matrix (see movie). The xanthan films impregnated with MPs, were gently dried with paper tissues, freeze-dried for ICP analysis or dried in oven at $(50 \pm 1)^\circ\text{C}$ overnight for SEM, STEM and SQUID analyses.

The *isoelectric points* (pI) of MP-US and MP-Heat were determined $(24 \pm 1)^\circ\text{C}$ by potentiometric titration (pHmeter Digimed DM20, São Paulo, Brazil). Aliquots of either 0.025 M HCl or 0.06 M NaOH (typically 20 μL) were added to the 0.1 g of magnetite dispersed in 30 mL KNO_3 0.1 mol.L⁻¹ (electrolyte) and stirred 10 minutes in order to change the initial solution

pH, which was measured with a glass electrode. The amount of protons (Q) in moles consumed or released by each gram of adsorbent was calculated using equation 1 (Puziy et al., 2004):

$$Q = \left(\frac{V_0 + V_t}{m} \right) \times ([H^+]_i - [OH^-]_i - [H^+]_e + [OH^-]_e) \quad (1)$$

where V_0 and V_t are the volumes of background electrolyte and added titrant, respectively, and m is the mass of the adsorbent. The subscripts “ i ” and “ e ” refer to initial and equilibrium concentrations, respectively. The initial proton concentration was calculated from the amount of added titrant. The equilibrium proton concentration was calculated from the measured pH. Background electrolyte KNO_3 0.1 mol.L⁻¹ yielded similar titration curves.

Colloidal stability tests. The stability of the MP-US and MP-Heat dispersions was studied using a separation analyzer LUMiReader®414 Separation Analyzer (L.U.M. GmbH, Germany) at (25 ± 2) °C. The phase separation behavior was monitored by the SEP View 4.01 software, which registered the normalized integral light transmission as a function of time. Glass cuvettes 80 mm long and optical path of 10 mm used for the experiments. At the beginning the dispersion is homogeneous and the transmitted light through the cuvette is very low. As time goes by the particles start to sediment, accumulating at the bottom of the cuvette and increasing the transmission of light through the upper liquid. The transmitted light along the cuvette is integrated and shown as a function of time.

X-Ray Powder Diffraction. X-ray diffractograms of freeze-dried MPs were obtained in a Rigaku equipment ($\lambda = 0.154$ nm), in the 2θ range of 30° to 65°, with intervals of 0.02° and 10 s accumulation. The diffractograms were analyzed with a CSM (*Crystallographica Search-Match*) software.

Infrared Spectroscopy. IR spectra of dried MPs were obtained with KBr pellets (3 mg freeze-dried MP per 150 mg KBr, thickness 1 mm), 4 cm⁻¹ resolution in a Bohmen MB100 spectrometer.

Magnetization Properties. A superconducting quantum interference device (SQUID) magnetometer (model MPMS of Quantum Design) was used to analyze the magnetic properties of MP-US, MP-Heat and MP-US impregnated in the xanthan networks. A known amount of sample was packed in a capsule made of acetate cellulose and inserted in a polyethylene straw as a sample holder. The magnetization measurements as a function of the applied magnetic field were recorded at 5 K and 300 K, with a maximum magnetic field of 70 kOe. The magnetization zero-field cooling (ZFC) and field cooling (FC) curves were obtained as follows. The sample

was mounted in the SQUID at room temperature and then cooled down to 5 K at zero field. The magnetization was thus measured in the presence of a dc field of 50 Oe while the temperature was raised up to 300 K, yielding the ZFC curve. Subsequently the sample was cooled down again to 5 K but maintaining the applied field (FC curve).

Atomic force microscopy (AFM) was used to estimate the mean size of MP-US adsorbed onto a xanthan monolayer. Xanthan-covered Si wafers (Carbohydrate Polymers 2012-Dario) were immersed in the MP-US dispersion for one hour at (24 ± 1) °C. After that the samples were rinsed in distilled water three times and gently dried under a stream of N₂. The surfaces were analyzed with a PICO SPM-LE (Molecular Imaging) microscope in the intermittent contact in air at room temperature, using aluminum coated silicon cantilevers with resonance frequency close to 310 kHz. Scan areas of 600 nm X 600 nm were obtained with a resolution of 512×512 pixels.

Ellipsometry was used to determine the thickness of the adsorbed MP-US layer onto xanthan films. Ellipsometric measurements were performed in air using a vertical computer-controlled DRE-EL02 ellipsometer (Ratzeburg, Germany). The angle of incidence was set at 70.0° and the wavelength, λ , of the He-Ne laser was 632.8 nm. For the data interpretation, a multilayer model composed by the substrate, the unknown layer and the surrounding medium should be used. Then the thickness (d_x) and refractive index (n_x) of the unknown layer can be calculated from the ellipsometric angles, Δ and Ψ , using the fundamental ellipsometric equation and iterative calculations with Jones matrices (Azzam and Bashara, 1979):

$$e^{i\Delta} \tan \Psi = R_p/R_s = f(n_k, d_k, \lambda, \phi) \quad (2)$$

where R_p and R_s are the overall reflection coefficients for the parallel and perpendicular waves. They are a function of the angle of incidence ϕ , the wavelength λ of the radiation and of the refractive index and the thickness of each layer of the model, n_k , d_k .

First of all, the thickness of the SiO₂ layers was determined in air, considering the refractive index for Si as $\tilde{n} = 3.88 - i0.018$ (Palik, 1985) and its thickness as an infinite one, for the surrounding medium (air) the refractive index was considered as 1.00. For the native SiO₂ layer, the refractive index was set as 1.462 (Palik, 1985) and the thickness was determined as (1.9 ± 0.1) nm. For the xanthan layer, the index of refraction was 1.52 (Dario et al., 2011) and the thickness was determined as (3.0 ± 0.5) nm. The thickness (d_{iter}) and index of refraction (n_{iter}) of magnetite layer were determined independently by iterative calculations (Azzam and Bashara, 1979).

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a Spectro Smart Analyzer Vision equipment (SPECTRO Analytical Instruments GmbH, Germany), yielded the amount of iron in the magnetite impregnated xanthan films.

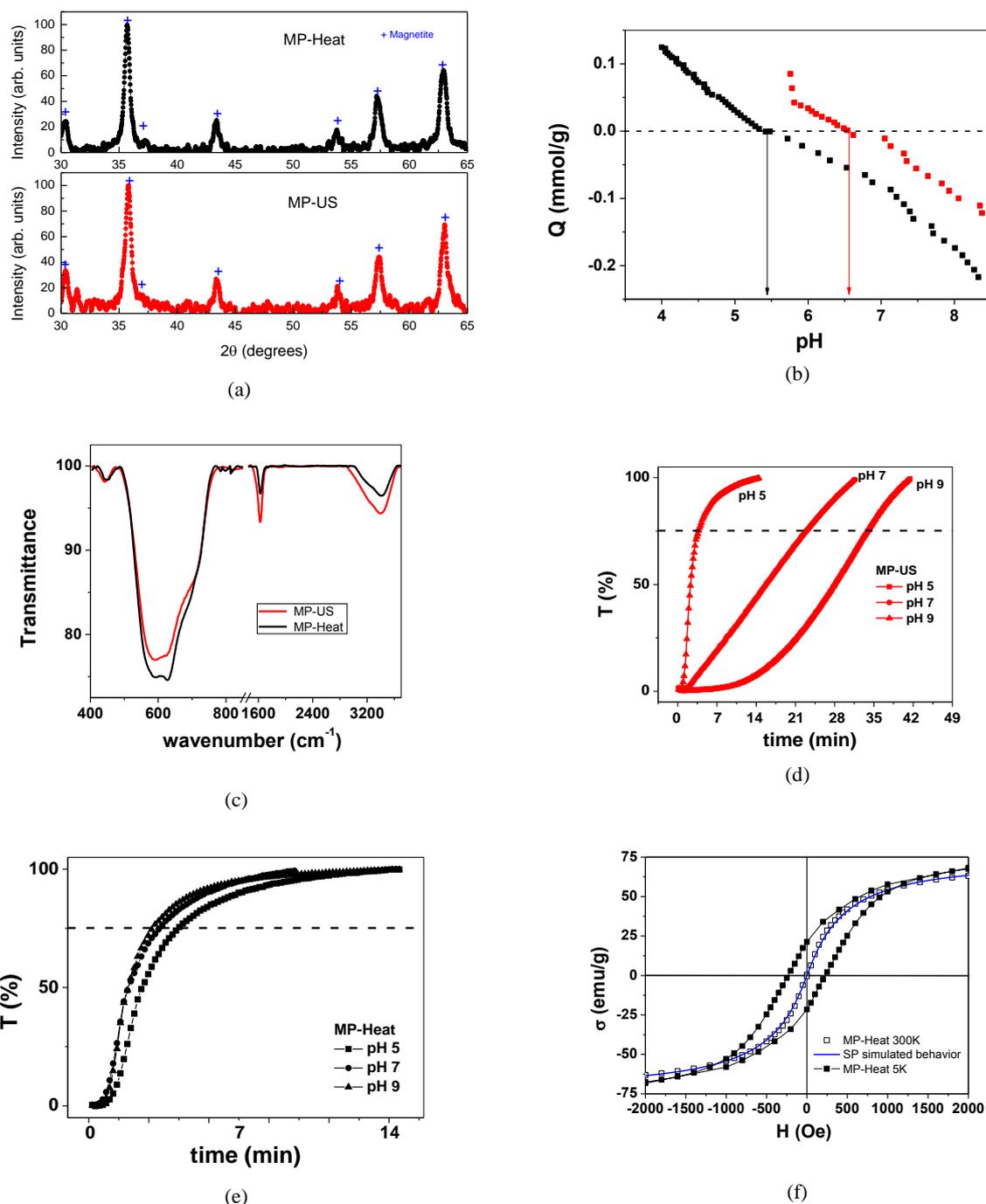
The quantification of Ca^{2+} ions in the cells was performed as follows. Cell culture was trypsinized and centrifuged. Cellular pellet was lysed with 200 μL of triton x100 solution. Resultant solution was acidified with 50 μL of HCl and diluted with 10 mL of deionized water prior to inject on ICP-AES apparatus. In average Ca^{2+} ions concentration in cells grown in the presence of ESMF was 9.84 ppm, while in the cells grown in the absence of ESMF was 9.31 ppm.

Scanning electron microscopy (SEM) technique was used in the morphological investigation of the MP-US xanthan composite films. An available FEI Inspect F50 high resolution scanning electron microscope (LNNano-CNPEM) was used for image acquisition. Samples were prepared by tearing small pieces of composite films already used in the magnetization measurements. The small slivers thus obtained were sandwiched within oyster TEM grids, as shown in Figure ESI-4. Secondary (SEI) and backscattered electron images (BEI) were acquired from both the upper surface (Figure ESI-3) and from the interior (cross-section) of the films. Scanning electron images were acquired in transmission (STEM) at the edges of the films lower surface.

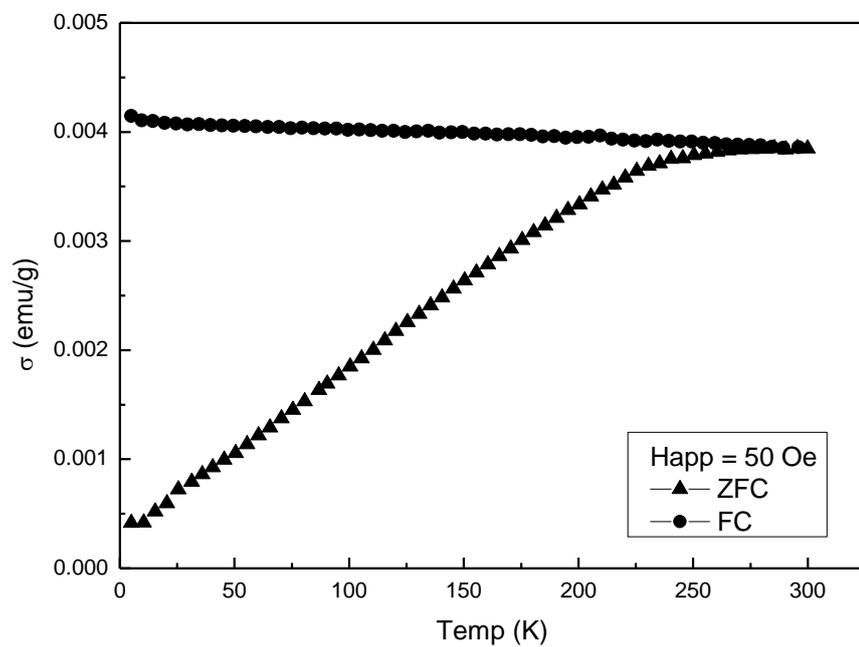
Cellular Adhesion and Proliferation Assay. Dry hydrogels samples were cut in round sheet scaffolds format (diameter 12mm) and exposed to UV light during 15 min each side for sterilization. This samples were placed in cell culture plates (Costar, Corning, NY, USA) and wetted with DMEM medium supplemented with 10% of fetal bovine serum (FBS) containing penicillin (100 IU mL^{-1}), streptomycin (100 mg mL^{-1}) and amphotericin B (50 mg mL^{-1}) for 24 h before cell seeding. The medium was removed and 3T3-L1 fibroblasts were seeded at a density of $4.4 \times 10^4 \text{ cells cm}^{-2}$ in 25 μL of supplemented DMEM. After 2 h of incubation, 250 μL of culture medium (supplemented DMEM) was added. The samples were incubated at 37 °C and 5% CO_2 atmosphere and the complete media was refreshed every 2 days. For SEM studies, membranes were washed with phosphate buffered saline (PBS), fixed with formalin (10%) for 15 min, dehydrated in aqueous ethanol solution by washing 10 min with concentrations of 25, 50, 70, 90, 95, and 100 vol% ethanol and dried. For adhesion and proliferation assay, scaffolds were rinsed once with PBS solution and placed in a new well plate containing 300 μL of DMEM with MTT (0.5 mg mL^{-1}) in every well. After 3 h, solution was removed and 1mL of DMSO was added to each well to dissolve MTT-formazan crystals. Solutions were diluted to

6mL to respect Lambert-Beer linearity. Aliquots (500 μ L) were taken in order to measure the absorbance at 570 nm (Shimadzu Multispec 1501). MTT assay were done in quadruplicate.

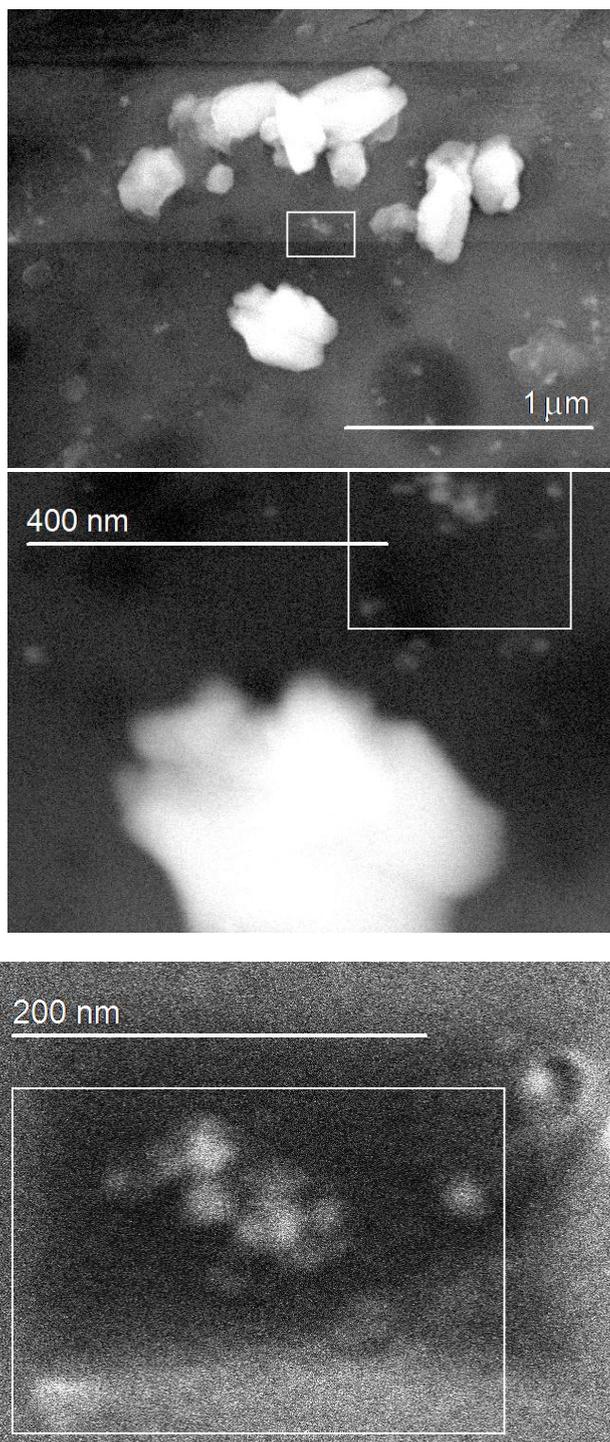
Dynamic Light Scattering (DLS) measurements were performed in a commercial instrument Zetasizer NanoZS (Malvern, UK). A He-Ne laser was used as a light source with wavelength $\lambda=633$ nm. Concerning the DLS experiments, the intensity of light scattered was recorded at an angle of 90° with an avalanche photodiode detector. We used the Zetasizer Software 6.2 (provided by Malvern) to determine the particle size distribution. In few words, the software uses the correlation function to obtain the distributions of the decay rates, and hence, the apparent diffusion coefficients. Finally, the distributions of the hydrodynamic radius of the scattering particles in solution are calculated via Stokes-Einstein equation. The measurements were performed for stock dispersions of MP-Heat and MP-US at pH 9 after filtering through a 0.45 μ m Millipore filter. In the case of MP-Heat no scattering could be detected by the equipment, indicating the absence of particles smaller than 450 nm in the filtrate. In the case of MP-US, populations with mean diameter D of 18 nm, 164 nm and 258 nm were detected, with polydispersity indices varying from 0.53 to 0.89.



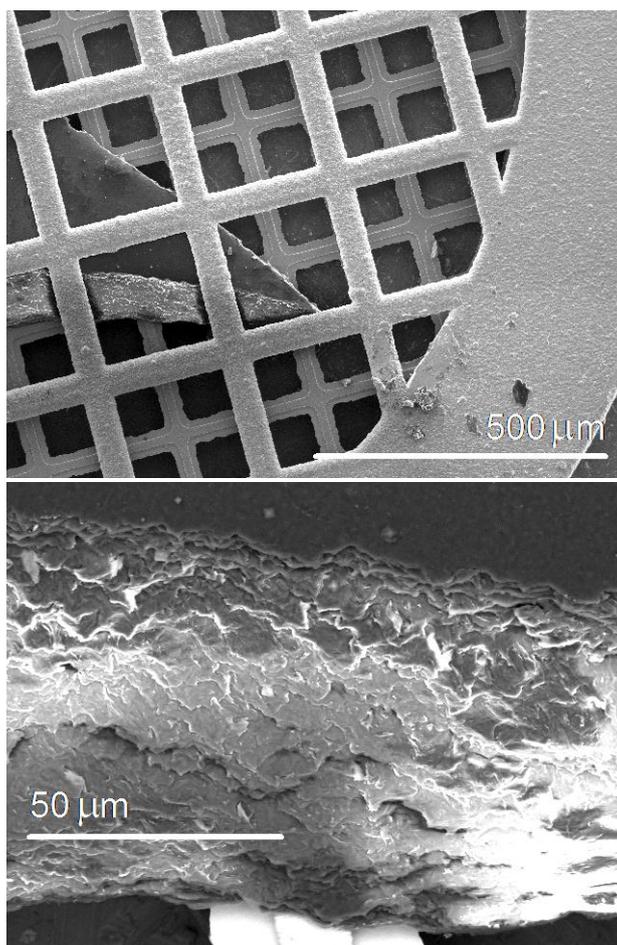
ESI-1. Characterization of MP-US (red) and MP-Heat (black). (a) X-ray diffractograms. (b) Amount of protons (Q) in moles consumed or released by each gram of adsorbent as a function of pH. (c) FTIR spectra. Integral transmittance as a function of time determined at pH 5, 7 and 9 for dispersions of (d) MP-US and (e) MP-Heat. (f) Hysteresis loops measured at 300 K and 5 K for MP-Heat, respectively (blue solid line corresponds to simulations of superparamagnetic behavior).



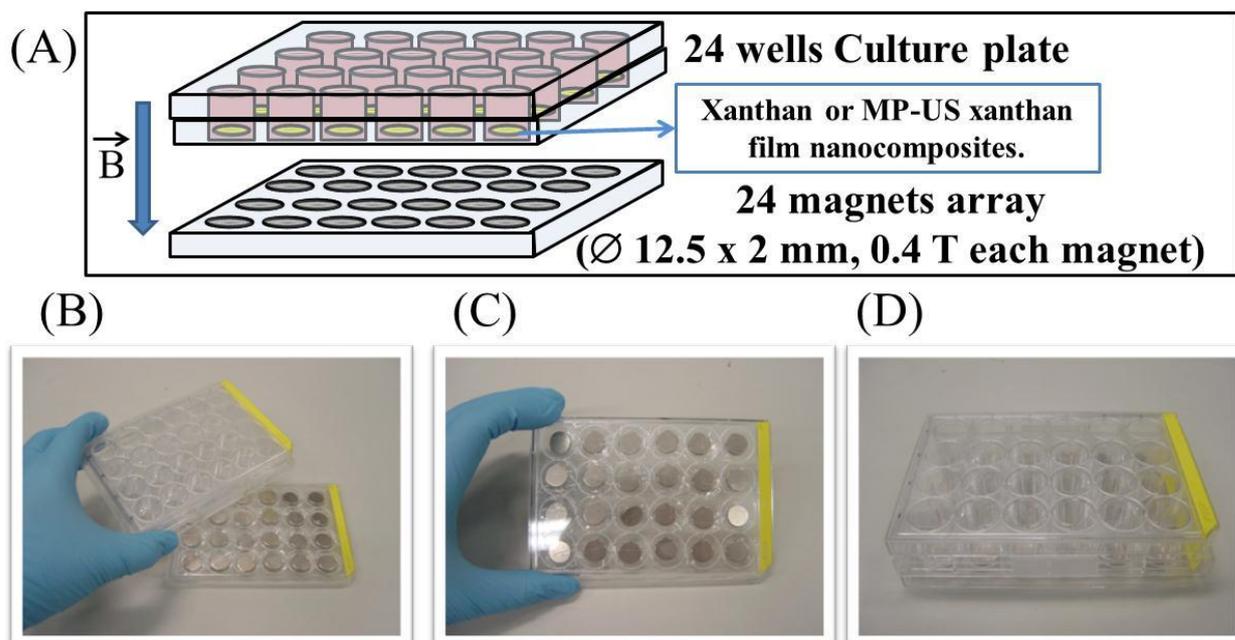
ESI-2. Coercivity as a function of temperature for the determination of blocking temperature for MP-US xanthan nanocomposite films.



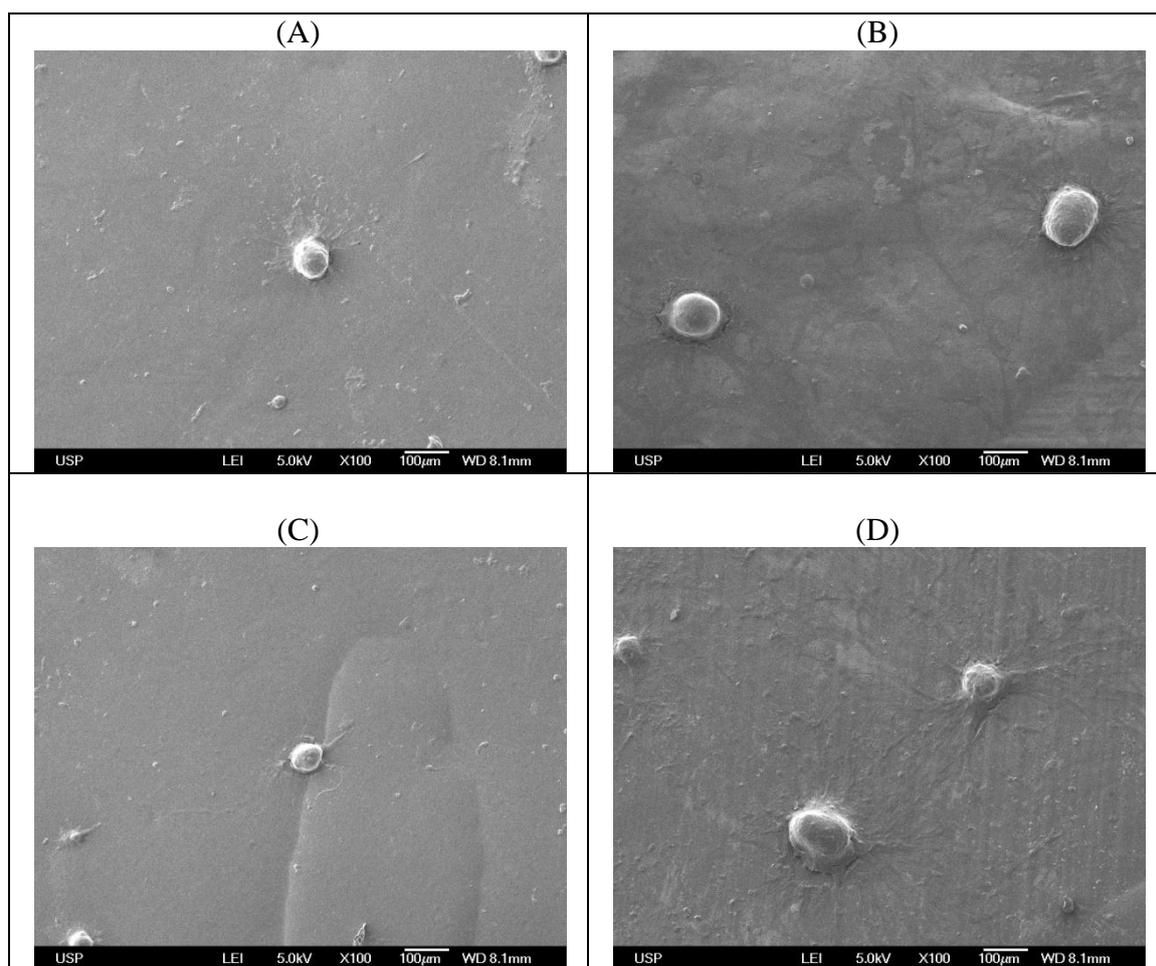
ESI-3. Sequence of SEM images acquired with increasing magnifications of a MP-US nanocomposite surface region. The white squared area shows individual magnetite nanoparticles adsorbed on the film surface, along with larger particles.



ESI-4. SEM images of the cross-sectional sample of the MP-US nanocomposite film. The higher magnification image shows typical nanocomposite regions available for morphological investigation, namely the upper surface, the interior and edges of the lower surface of the film.



ESI-5. Cell culture plates with magnets array used for cellular adhesion and proliferation assay cellular. (A) Schematic representation of the set. Photographs of the set, showing the magnets array (B), detail of the magnetic array (C) and of assembled set (D).



ESI-6. SEM micrographs of fibroblasts culture on bare xanthan and MP-US xanthan nanocomposites films, after 14 days incubation. (A) Xanthan and (B) MP-US xanthan hybrid films without ESMF exposition; (C) xanthan and (D) MP-US xanthan hybrid films with ESMF exposition.

References

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