# Supporting information

## Magnetic and radio-labeled bio-hybrid scaffolds to promote and track in vivo the progress of bone regeneration

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LIST OF ACRONYMS:

| ACRONYM | DESCRIPTION |
|---|---|
| SPECT | Single Photon Emission Computed Tomography |
| CT | Computed tomography scan |
| MRI | Magnetic Resonance Imaging |
| [$^{99m}$Tc]Tc-MDP | Technetium (99mTc) medronic acid, a commercial radiotracer |
| TEPSA | 3-(Triethoxysilyl)propylsuccinic anhydride |
| SPIONs | Superparamagnetic iron oxide nanoparticles |
| TEPSA-SPIONs | Superparamagnetic iron oxide nanoparticles coated with TEPSA |
| nMNPs | Naked SPIONs |
| MNPs | TEPSA-SPIONs activated by EDC/sulfo-NHS coupling |
| HyS | Hybrid scaffold obtained thorough the biomineralization process in which hydroxyapatite is nucleated on collagen fibers |
| (nMNPs)HyS | Hybrid scaffold functionalized with naked SPIONs before biomineralization process |
| (MNPs)HyS | Hybrid scaffold functionalized with activated TEPSA-SPIONs before biomineralization process |
| Hy(nMNPs)S | Hybrid scaffold functionalized with naked SPIONs during biomineralization process |
| Hy(MNPs)S | Hybrid scaffold functionalized with activated TEPSA-SPIONs during biomineralization process |
| HyS(nMNPs) | Hybrid scaffold functionalized with naked SPIONs after biomineralization process |
| HyS(MNPs) | Hybrid scaffold functionalized with activated TEPSA-SPIONs after biomineralization process |

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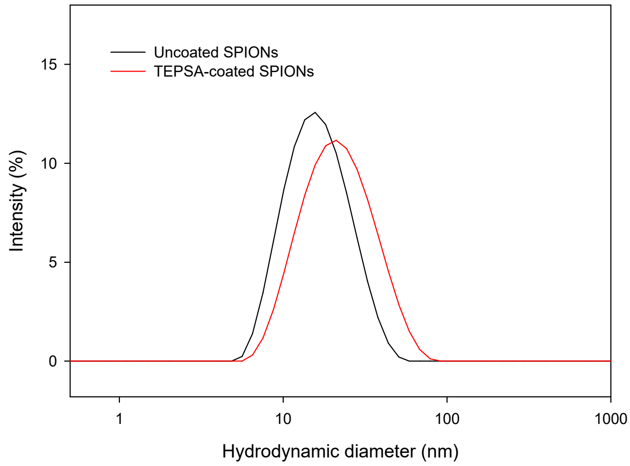



**Figure S1.** Size distribution in intensity as measured by photon correlation spectroscopy (PCS) of uncoated (black line) and TEPSA-coated NPs (red line).

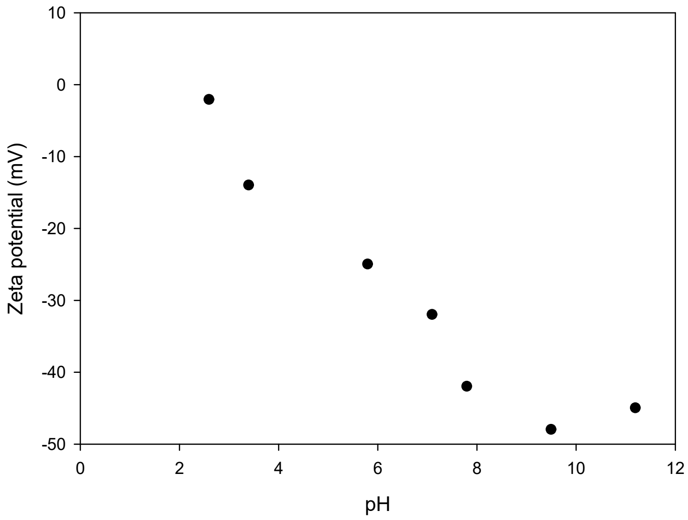



**Figure S2.** ζ-Potential measurements vs pH of TEPSA-coated nanoparticles.

**Figure S3.** Image representing the third preparation condition in which the hybrid composite was previously prepared and subsequently soaked in a solution containing the naked nanoparticles (nMNPs). In this case the resulting scaffold was visibly inhomogeneous due to precipitation of nMNPs during drying.

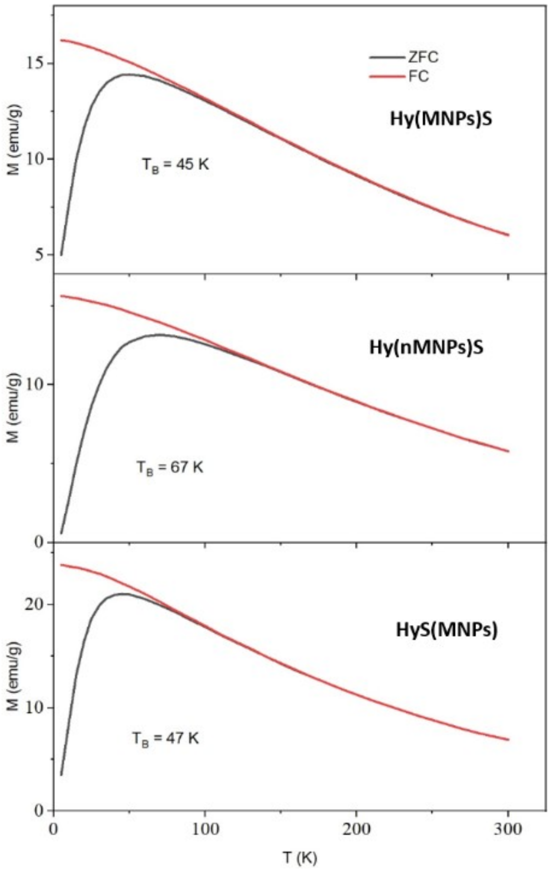



**Figure S4.** ZFC-FC curves of the samples at 100 Oe.

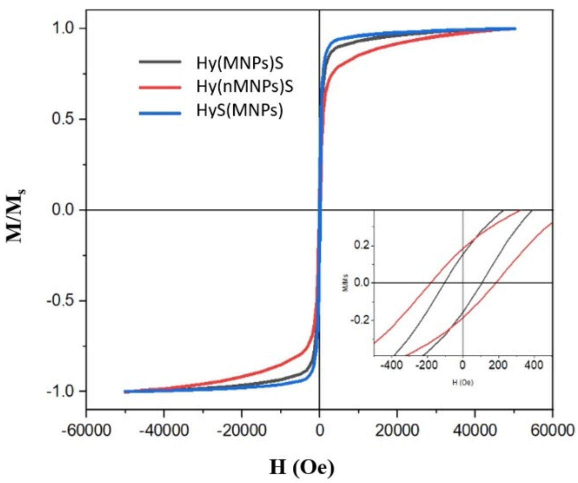
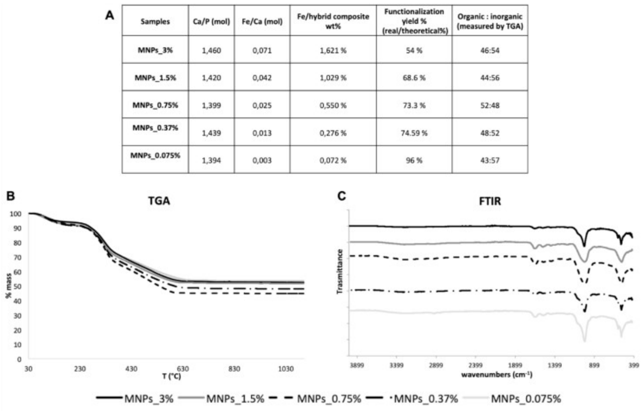



**Figure S5.** Normalized hysteresis loops at 5 K of the three samples. The inset shows a zoom at low field of samples Hy(MNPs)S and Hy(nMNPs)S



| Samples | Ca/P (mol) | Fe/Ca (mol) | Fe/hybrid composite wt% | Functionalization yield % (real/theoretical%) | Organic : inorganic (measured by TGA) |
|---|---|---|---|---|---|
| MNPs_3% | 1,460 | 0,071 | 1,621 % | 54 % | 46:54 |
| MNPs_1.5% | 1,420 | 0,042 | 1,029 % | 68.6 % | 44:56 |
| MNPs_0.75% | 1,399 | 0,025 | 0,550 % | 73.3 % | 52:48 |
| MNPs_0.37% | 1,439 | 0,013 | 0,276 % | 74.59 % | 48:52 |
| MNPs_0.075% | 1,394 | 0,003 | 0,072 % | 96 % | 43:57 |



**Figure S6.** A) Chemical composition of the MNPs_3%; MNPs_1.5%; MNPs_0.75%; MNPs_0.37%; MNPs_0.075% obtained from ICP analysis; B) Thermal decomposition profile (TG) of the MNPs_3%; MNPs_1.5%; MNPs_0.75%; MNPs_0.37%; MNPs_0.075%, the weight loss in % of mass is used to determine the organic/inorganic ratio of the different samples; C)FTIR spectra of the MNPs_3%; MNPs_1.5%; MNPs_0.75%; MNPs_0.37%; MNPs_0.075%.

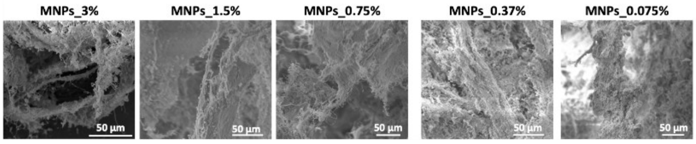



**Figure S7.** Scanning electron microscopy images of composites labeled with different amounts of MNPs (MNPs_3%; MNPs_1.5%; MNPs_0.75%; MNPs_0.37%; MNPs_0.075%).

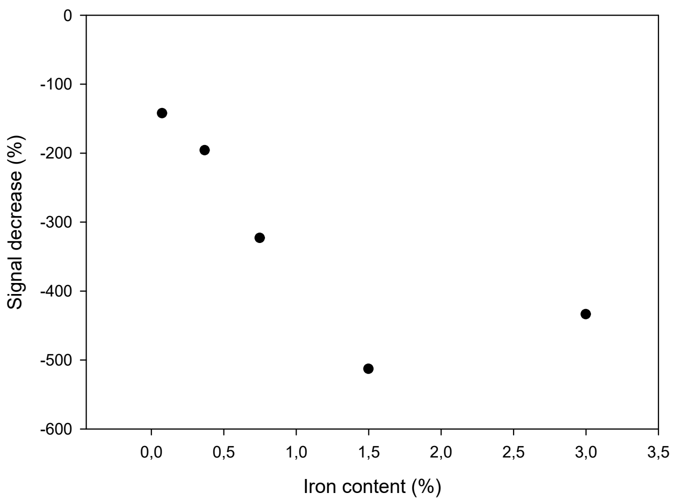
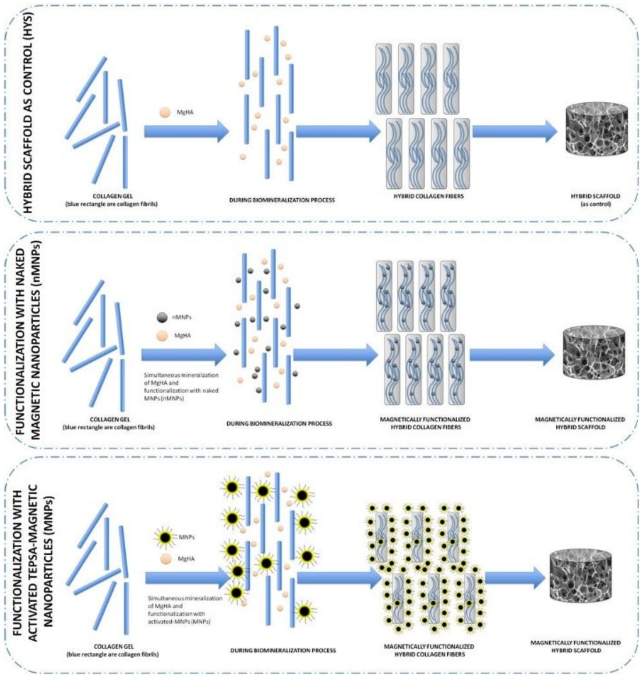



**Figure S8.** Decrease of the signal intensity (as expressed as the ratio between the signal of the magnetic matrix and the signal of the unlabelled scoffold) for the Hy(MNPs)S scaffolds regarding to their iron content.




**Scheme S1**. Schematic illustration of a hypothesized mechanism about magnetic functionalization dependent on the presence of i) an activated shell (yellow ring) linking on the collagen surface (MNPs) or ii) naked nanoparticles (black core) being entrapped into collagen fibers during self-assembling of fibers (nMNPs).