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Supporting Information

Monitoring tissue implants by field-cycling ^{1}H-MRI via the detection of changes in the ^{14}N-quadrupolar-peak from Imidazole moieties incorporated in the smart scaffold material

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1. Synthesis of oligo-His-PLGA-S and oligo-His-PLGA-L

Chemicals. All solvents and reagents were used as supplied. The (1-[Bis (dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium3-oxid hexafluorophosphate) (HATU), dimethylformamide (DMF; peptide synthesis grade), acetonitrile (HPLC grade) diisopropylethylamine (DIPEA), piperidine, triisopropylsilane (TIS) and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich.

Measurements: ^{1}H-NMR spectra were recorded at 310 K with a Bruker AVANCE 600 spectrometer operating at 14 T (corresponding to 600 MHz ^{1}H Larmor frequency), equipped with an inverse Z-gradient 5 mm BBI using DMSO as solvent.

Synthesis of the CGGH$_{15}$βA (=oligo-His) peptide and synthesis of Maleimide-PEG$_2$-NH$_2$: the automated synthesis of peptide CGGH$_{15}$βA and preparation of Maleimide-PEG$_2$-NH$_2$ linker were performed according to a previously described procedure. [34]

Synthesis of PLGA-PEG$_2$-Mal-S and PLGA-PEG$_2$-Mal-L: PLGA-PEG$_2$-Mal-S was synthetized starting from PLGA Resomer® RG 502 H, PM= 12800. 1.0 g of PLGA was dissolved in 30 mL of dry CH_3CN in a 25 mL flask. 0.056 mL (0.32 mmol) of DIPEA and HATU (0.076 g, 0.2 mmol) were added to the flask. After 5 min Maleimide-PEG$_2$-NH$_2$ (0.060 g, 0.2 mmol) dissolved in CH_3CN (2 mL) is added dropwise and then the mixture was stirred at room temperature for 6 h under N_2 atmosphere. The solvent was then removed under vacuum and the residue was dissolved with chloroform and washed three times 1M HCl and then several times with brine and water. The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give product in almost quantitative yield. PLGA-PEG$_2$-Mal-L was prepared following the same procedure starting from PLGA Resomer® RG 504 H, PM= 52900 (yield=85%). ^{1}H

NMR dmso-d6, 600 MHz) δ 7.03 (s, maleimide protons), 5.31-5.0 (m, CH lactide proton), 4.98-4.85 (m, CH2 glycolide proton), 1.54 -1.46 (m, CH3 lactide proton).

Synthesis of oligo-His-PLGA-S and oligo-His-PLGA-L: To a solution of PLGA-PEG_2-Mal-S (1.0 g, 0.08 mmol) in 4 mL of anhydrous DMF, a solution of $CGGH_{15}$βA peptide (0.4 g, 0.16 mmol) in DMF (0.5 mL) and DIPEA (56 μL, 0.32 mmol) were added. The resulting mixture was magnetically stirred at room temperature for 8 h under nitrogen atmosphere. The desired oligoHis-PLGA-S was obtained by precipitation from cold diethyl ether/methanol 1:1 (50 mL) that was collected by filtration. The precipitate was then washed with a large amount of methanol to remove traces of uncoupled oligo-His and dried under vacuum (yield 80%). Oligo-His-PLGA-L was prepared following the same procedure starting from 0.8 g of PLGA-PEG_2-Mal-L (yield 70%). The products were analyzed by ^{1}H-NMR-spectroscopy (600 MHz, dmso-d6, **Figure S1** and **Figure S2**). For both of the two conjugates, the appearance of peptide peaks (Hα protons at 4.6 ppm and Hβ protons at 3.0 and 2.9 ppm, H4 and H2 protons of imidazole at 6.9 and 8.4 ppm, respectively) indicates the successful coupling of oligo-His to PLGA. The molar ratio of oligo-His to the copolymer was determined by the integrations of the peptide peak at 4.6 ppm and the peak of the –CH groups of polylactic acid at 5.2 ppm, respectively. The result indicated a 1:1 molar ratio of PLGA to oligo-His for both conjugates**.**

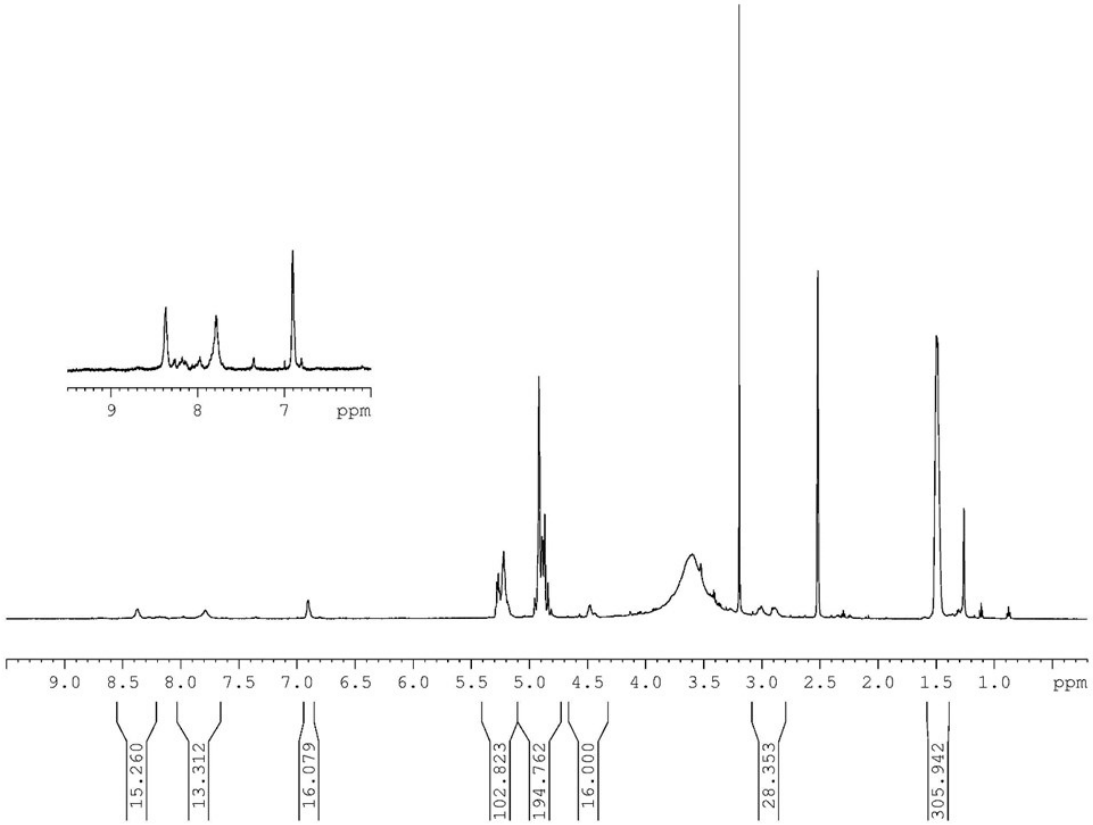


Figure S1. [1]H-NMR spectrum of oligo-His-PLGA-S.

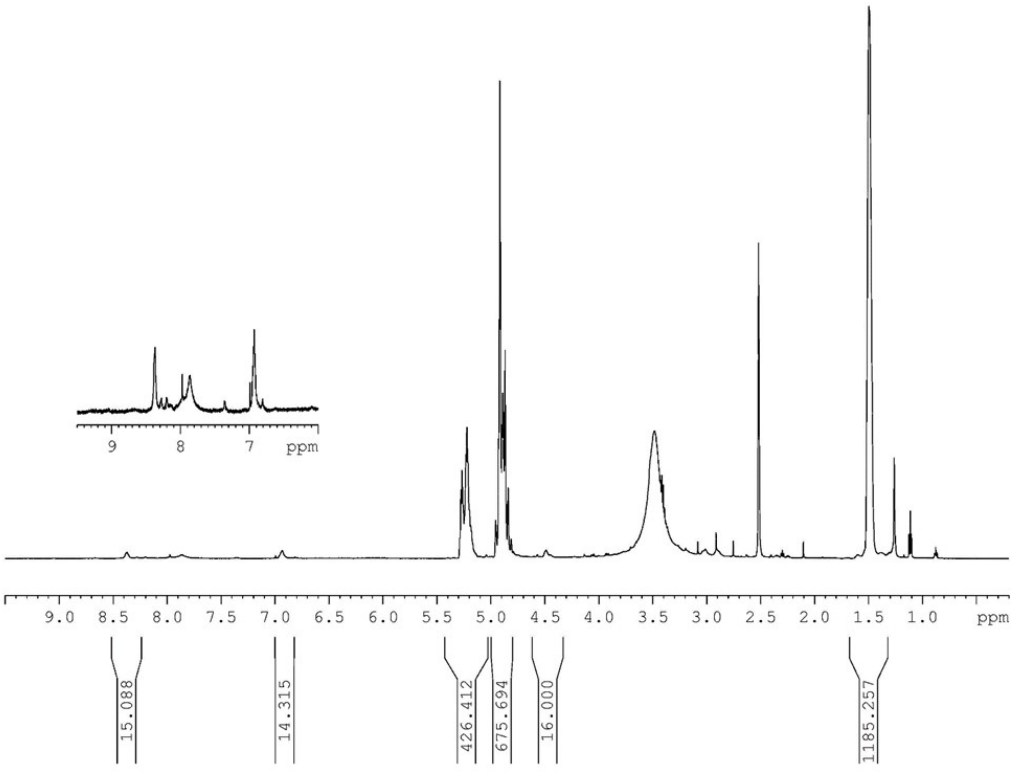


Figure S2. [1]H-NMR spectrum of oligo-His-PLGA-L.

2. Scaffold preparations

Scaffold preparations (100 mg ml^{-1}) were performed using several mixtures, mainly in terms of PLGA molecular weight ratio (**Table S1**). The scaffold was submitted to the NMRD profile acquisition recovered from the buffer after preparation (wetted) and/or after lyophilization and rehydration with a 70% of water (rehydrated). Each sample was evaluated considering the behavior at the transition from tretraglycol to aqueous solution (no, partial or good formation) and the height of the histidine peak (1.4 ± 0.2 MHz). The selected preparation was the last one reported in Table S1, in bold characters.

Table S1. *PLGA (50:50) molecular weights were the following: S= PLGA 7-17 kDa, L= PLGA 38-54 kDa and XL= PLGA 75-110 kDa, purchased from Sigma Aldrich.

Sample *	**Height of the His peak at 1.4±0.2 MHz (water content)**	**Scaffold formation**
S	n.d.	No formation
L	n.d.	good
L:oligo-His-S (75:25)	0.20 (wetted) 0.55 (rehydrated)	good
L:oligo-His-S (50:50)	0.06 (wetted)	partial precipitation
XL:oligo-His-S (50:50)	n.d. (wetted) 0.18 (rehydrated)	partial precipitation
oligo-His-L	broad (wetted) 0.20 (rehydrated)	good
oligo-His-L:oligo-His-S (75:25)	**0.24 (wetted) 0.71 (rehydrated)**	**good**

3. FFC in vitro scaffold imaging

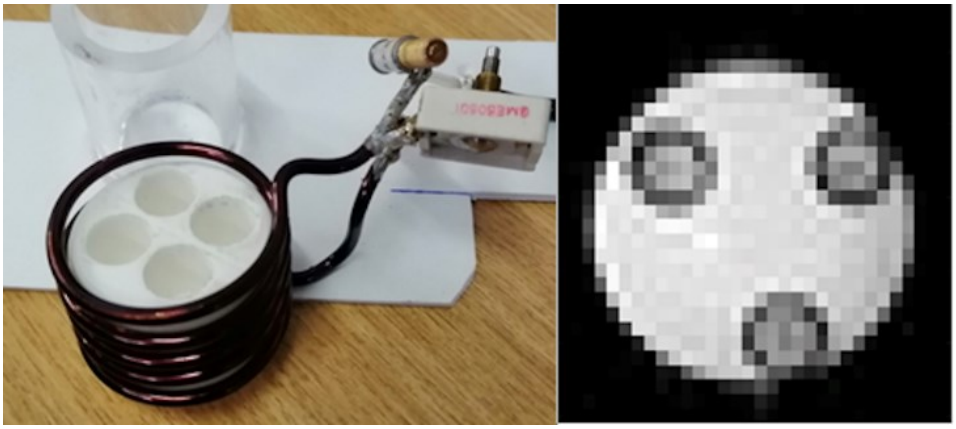
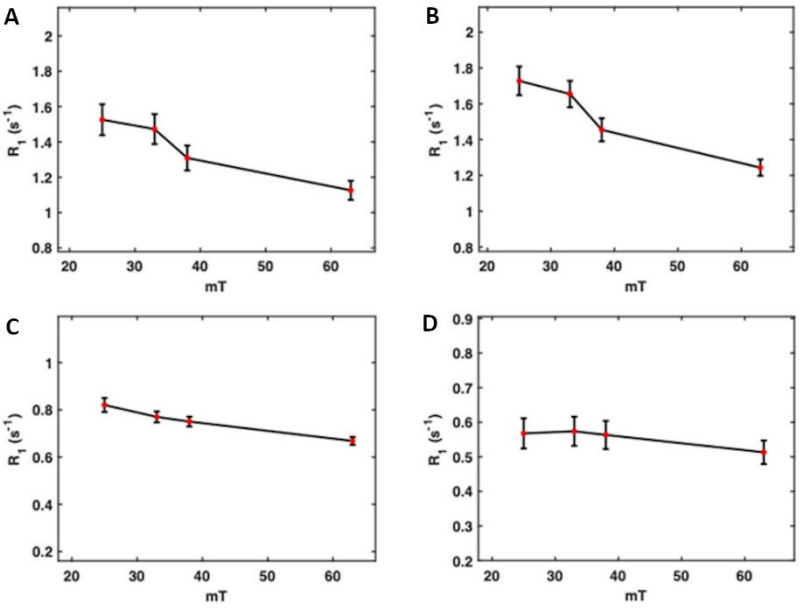


Figure S3. Left: 35 mm diameter RF coil used for the FFC imaging (see methods for details). Right: Proton density phantom image acquired at 0.2 T (proton Larmor frequency = 5 MHz) showing the two oligo-histidine scaffold samples and the control sample held in the agarose gel phantom.



FigureS4. Dispersion profiles obtained from the FFC images of: Oligo-His-PLGA scaffold sample at pH 6.4 (A) and pH 7.4 (B); agarose gel (C), PLGA control (D), The error bars stand for 2-sigma. Note the change of amplitude in the region of the histidine peak, as well as the general change in R_1 values and general dispersion that increase markedly with the presence of the scaffold.

4.In vivo scaffold implantation

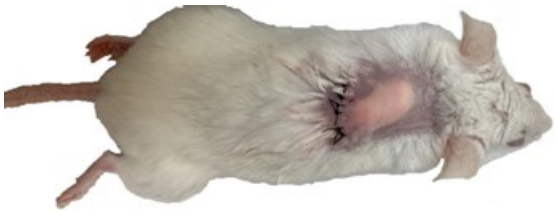


Figure S5. Representative image of scaffold implant. Oligo-His-PLGA or PLGA scaffold were implanted subcutaneously in the back of BALB/c mice.

4. Volumetric MRI analysis

Volumes of in vivo implanted oligo-His-PLGA- and PLGA-scaffolds were measured by acquiring multislice T_{2w} MRI at 7 T on a Bruker Avance 300 spectrometer. Regions of Interest (ROIs) were manually drawn for the scaffold analysis and volumes were accordingly measured for n = 12 mice for each sample group.

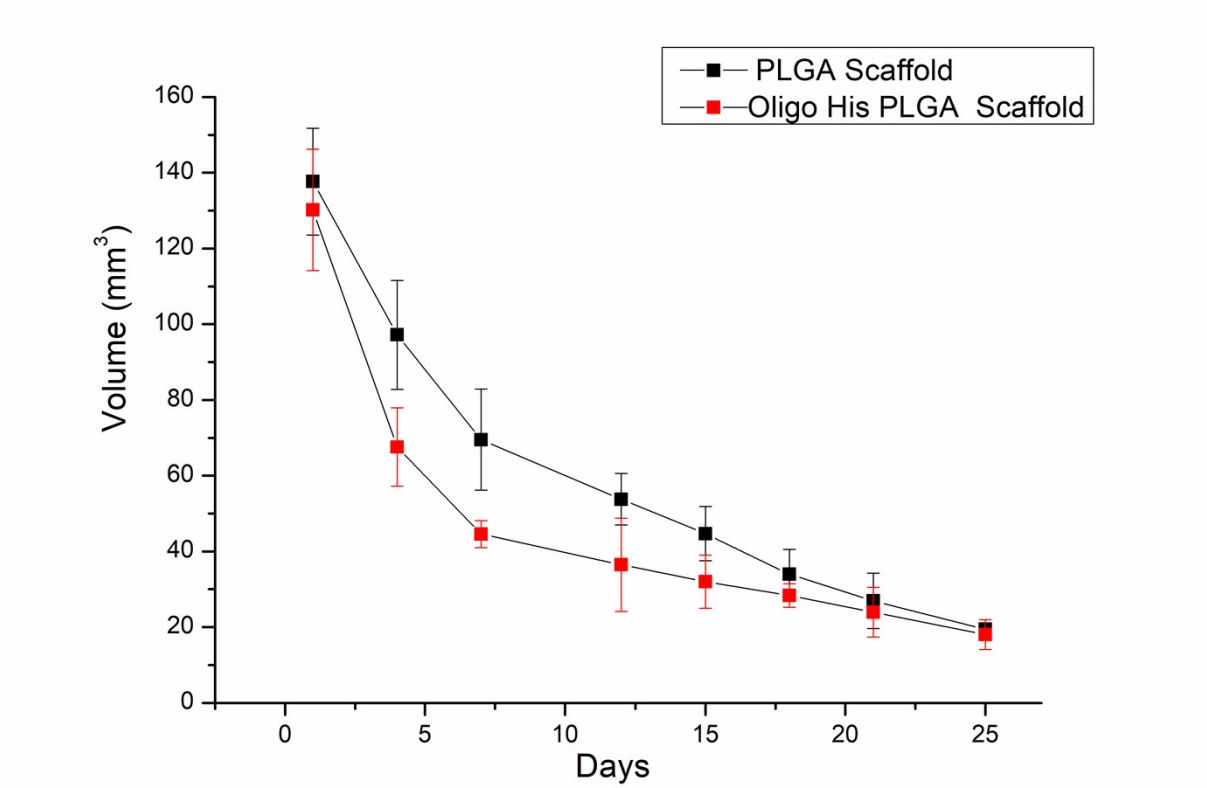


Figure S6. MRI volume measurements of the oligo-His-PLGA- and PLGA-scaffolds, from t = 1 to t = 25 days after scaffold implantation.

5. T_2 measurements

Transverse relaxation time (T_2) of *in vivo* implanted oligo-His-PLGA- and PLGA-scaffolds were measured at 7 T on a Bruker Avance 300 spectrometer (**Table S2**).

Table S2. MRI (7 T) T_2 measures of *in vivo* implanted oligo-His-PLGA- and PLGA-scaffolds

	T_2 (msec)				
Time	**1 day**	**7 days**	**12 days**	**19 days**	**25 days**
Oligo-His-PLGA Scaffold	55 ± 11	44 ± 2	40 ± 2	27 ± 6	21 ± 1
PLGA Scaffold	74± 5	94 ± 6	78 ± 6	88 ± 3	143 ± 4
Muscle	19 ± 2				

6. Masson's trichromic staining

For the visualization of collagen deposition by infiltrating mouse fibroblasts inside implanted scaffolds, trichromic staining was performed on 5 µm formalin fixed and paraffin-embedded sections using Trichrome Stain Kit (Abcam) according to manufacturer's instructions (**Figure S7**).

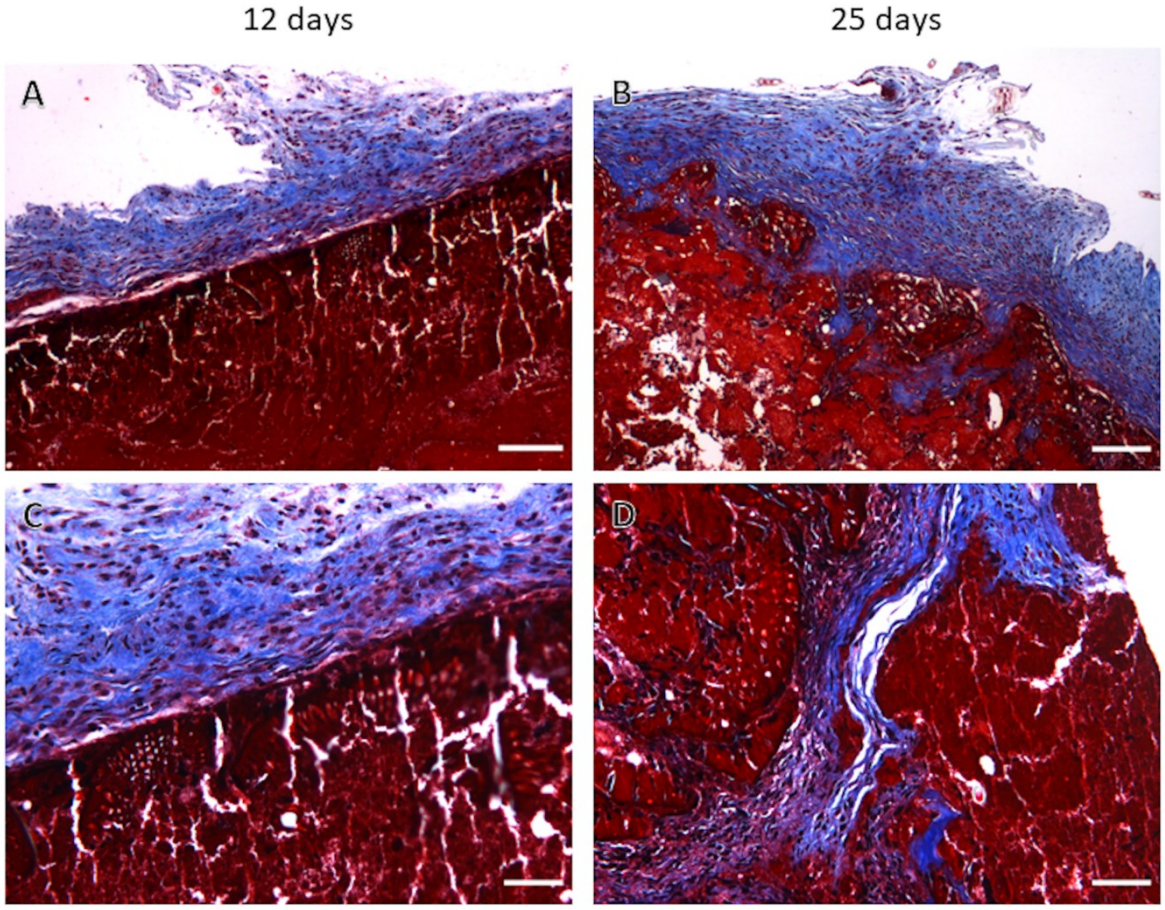


Figure S7. Masson's trichrome staining of oligo-His-PLGA scaffolds explanted after 12 days (A, C) and 25 days (B, D). Blue represents collagen, while the scaffold is marked in red. Scale bars = 50 µm (A, B), 100 µm (C, D).

7. Linear regression of ΔR_1 vs. the percentage of cells proliferating on the scaffold

ΔR_1 calculated at the peaks 1.39, 2.03 and 2.70 MHz of the scaffold NMRD profile were correlated with the % of cells proliferating on the scaffold quantified by the analysis of the H&E images (**Table S3).** ΔR_1 were calculated by considering the differences between the R1 values

measured at the following proton Larmor frequency values: 1.39 and 1.68 MHz, 2.03 and 4.4 MHz, 2.73 and 3.1 MHz, respectively.

Table S3. Linear regression of ΔR_1 *vs.* the % of cells proliferating on the scaffold.

Peak in the NMRD profile	**Intercept**	**Slope**	**Pearson (r)**	**Correlation**
1.39 MHz	5,57± 0,34	-0,055 ±0,011	-0,88	very strong
2.03 MHz	3,36± 0,55	-0,026±0,019	-0,46	moderate
2.70 MHz	4,24±0,47	-0,034±0,015	-0.62	strong