Novel quadrupolar peaks based contrast agents for monitoring tissue implants

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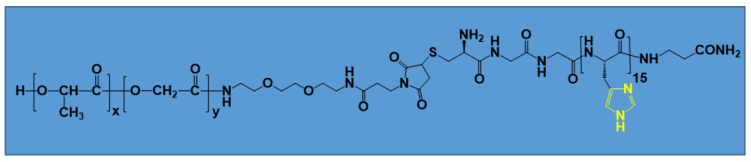
Introduction

Nowadays, one of the most important challenges in many medical fields is represented by the application of regenerative medicine approaches. To date there is an almost complete lack of methods for the rapid, non-invasive and repeated monitoring of tissue implants and new methods are needed to monitor cell status and polymer degradation under physiological conditions (temperature, saline, pH, enzymes etc.) thus allowing the physician to control, in real time, the transplanted scaffold status. This study aims at developing an innovative class of MRI contrast agents for Fast Field Cycling-MRI applications. They represent a completely new class of MRI contrast agents that display remarkable relaxation effects on tissue water protons. Their detection requires the acquisition of images at variable magnetic field strength as provided by Fast Field Cycling MRI (FFC-MRI) scanners. FFC is an innovative technology that allows detecting the quadrupolar cross-relaxation, appearing as peaks (QPs) in the $1/T_1$ dispersion profile completely invisible to conventional (fixed-field) MRI[1].

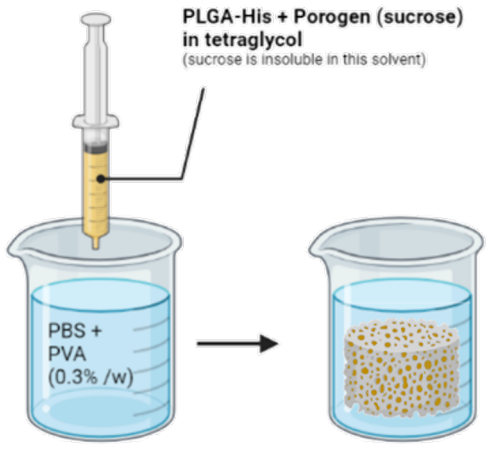
Results

PLGA scaffolds were prepared by dissolving PLGA conjugated with polyhistidine (n=15) in tetraglycol with glucose to create porous scaffolds.

histidine in polymeric form linked to poly lactic and glycolic acid (PLGA)



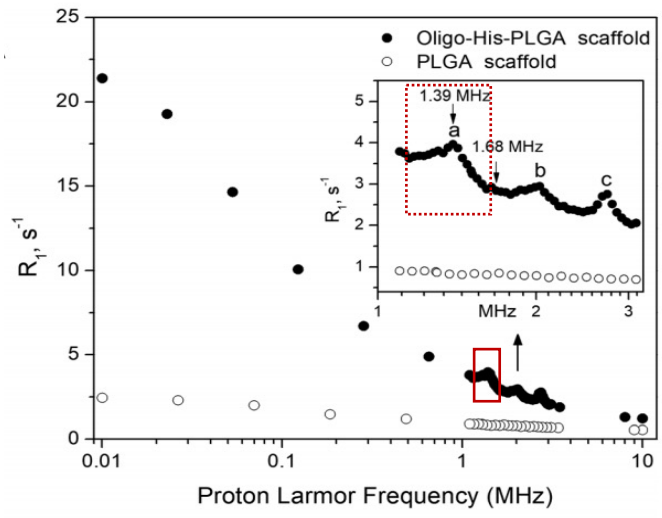
PLGA-polyHis



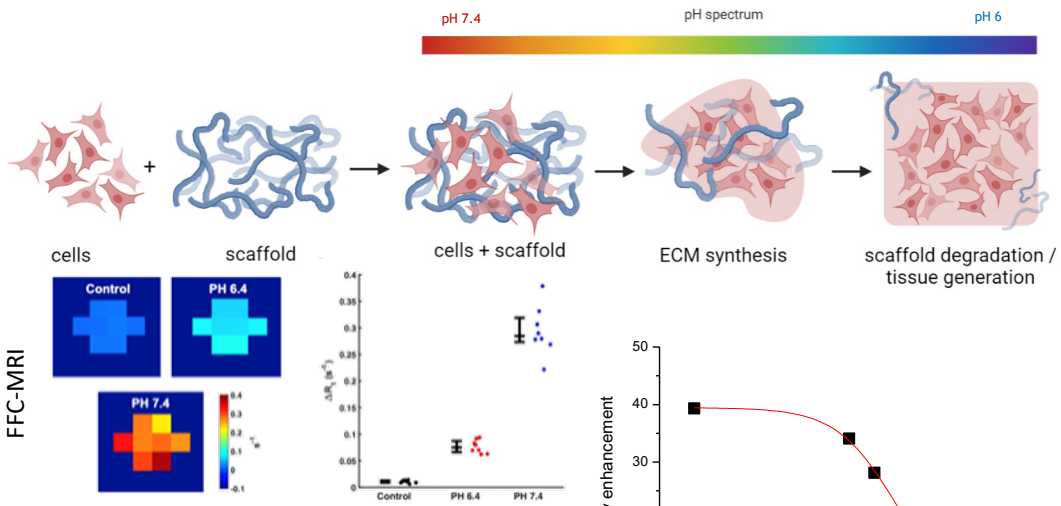
The mixture was then injected into PBS, where it precipitated by phase inversion to form a solid scaffold as the tetraglycol diffused into the PBS and allowed the porogen to be leached from the scaffold to form a porous structure.

The nuclear magnetic relaxation dispersion (NMRD) profile acquired on this biomaterial showed:

- The contrast is generated by the interaction of H_2O with quadrupolar ^{14}N of the imidazole group of histidine (peak at **1.39 MHz).**

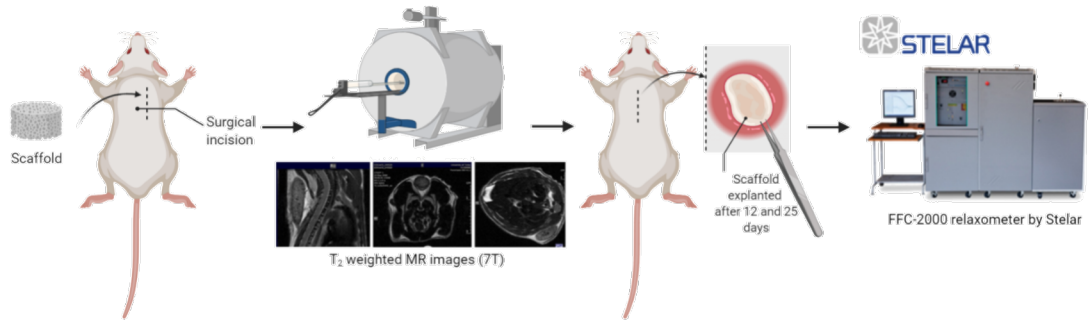


Synthetic tissue implants acts as a temporary substitute for extracellular matrices, providing an initial mechanical support for transplanted cells until the tissue can regenerate.

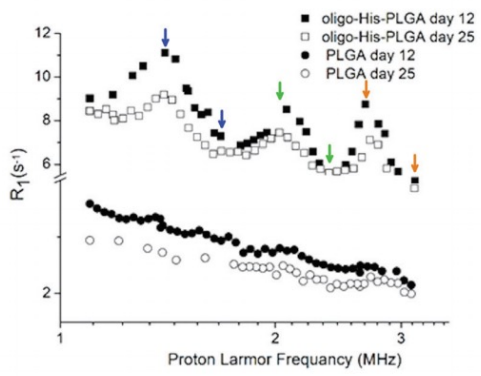


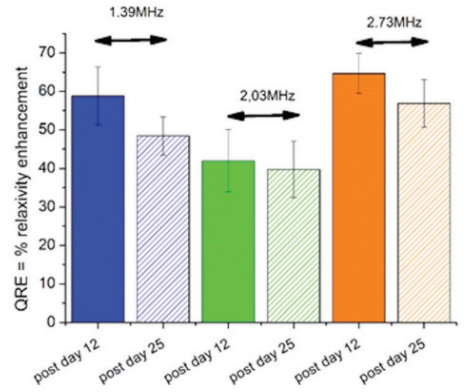
- The QPs are detectable only when the contrast agent is in a gelified or solid-like form, ie at pH>6.6, and above this value their intensity is pH dependent[2].
- Thanks to this pH-dependent behaviour, polyHis-scaffolds can be used to report on tissue pH changes.

Oligo-His-PLGA and PLGA (control) scaffolds were surgically implanted in the upper part of the back of Balb/c mice.



T_2-weighted MR images acquired 1 and 25 days after implantation showed that scaffolds are stably encapsulated in the subcutaneous region. Mice (n = 12 for each sample group) were euthanized 12 and 25 days after surgery; the scaffolds were explanted and analyzed by means of FFC-NMR and histology.



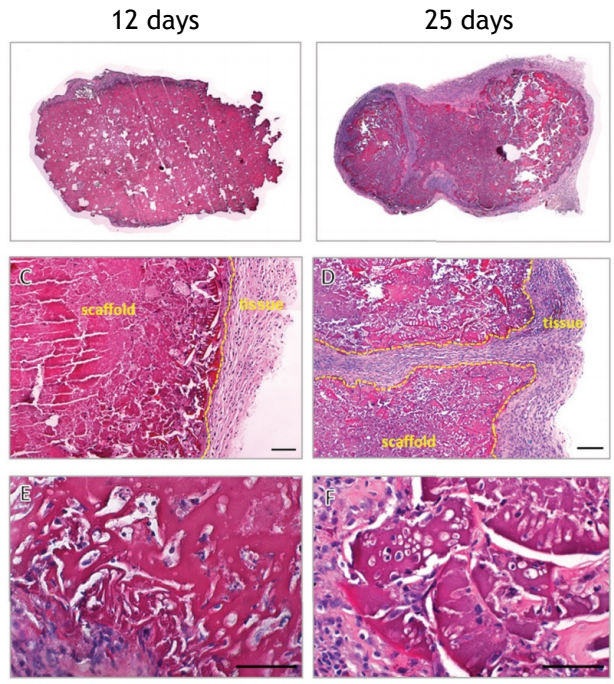


Scaffolds analyzed 25 days after implant showed reduced quadrupolar relaxation enhancements (QREs) with respect to 12 days:

- QRE decrease at 1.39 MHz of ca. 18%, probably due to pH change after cells' colonization.
- a less pronounced QRE decrease at 2.03 and 2.7 MHz (5.3 and 12% respectively) due to the contributions arising from proteins of the newly generated tissue that fall at these frequencies.

Haematoxylin and Eosin (H&E) staining of explanted scaffolds showed:

- 12 days: an initial cell invasion of the structures, with endogenous cells surrounding all the external surface of the scaffold.
- 25 days: cells colonize the entire scaffolds pores and the exogenous materials start to degrade.



Conclusions

- These sensors are based on oligo-histidine moieties that are conjugated to PLGA polymers representing the structural matrix for cells hosting scaffolds.
- The presence of ^{14}N atoms of histidine causes a quadrupolar relaxation enhancement at 1.39 MHz. This QP falls at a frequency well distinct from the QPs generated by endogenous semisolid proteins.
- The relaxation enhancement is pH dependent in the range 6.5–7.5, thus it acts as a reporter of the scaffold integrity as it progressively degrades upon lowering the microenvironmental pH.
- A good biocompatibility of the histidine-containing scaffolds is observed after its surgical implantation in healthy mice.
- In respect to the clinically used contrast agents this material has the advantage of generating contrast without the use of potentially toxic paramagnetic metal ions.

References

1. C. Gösweiner et al. Tuning Nuclear Quadrupole Resonance: A Novel Approach for the Design of Frequency-Selective MRI Contrast Agents, Phys. Rev. X 8, 021076. 1-20 (2018).
2. S. Geninatti Crich, S. Aime, R. Stefania, S. Baroni, M.R. Ruggiero, L. Broche, D. Lurie "Nuovi agenti di contrasto per risonanza magnetica per immagini" , Patent number: 102019000007647 (2019).