Magnetic Resonance MicroImaging of cell migration in porous biomaterial scaffolds designed for tissue engineering

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Purpose/Introduction

Tissue engineering aims to regenerate new biological tissues in order to replace diseased or injured tissues. Polymeric scaffolds[1] for cell seeding, cell growth and proliferation are often extremely sensitive to histological processing. Therefore 3D non-invasive and quantitative imaging methods are needed to study tissue-engineered constructs before and after implantation. This work investigates the use of high resolution MRI as a tool to monitor labeled cell distribution and migration within a porous scaffold.

Subjects and Methods

Human umbilical vein endothelial cells (Huvec-C, ATCC), magnetically labeled using anionic citrated maghemite nanoparticles[1] ([Fe]=0.05mM), were seeded in porous scaffolds prepared from polysaccharides[2]. Cells were cultured for 8 days and their migration within the scaffold was followed by micro-imaging using a high-temperature superconducting (HTS) surface coil[3] combined with a whole body 1.5 T magnet (Philips Achieva, CIERM Kremlin Bicêtre). The HTS coil operated at 77 K with an unloaded *Q* factor of 10,700 inside the magnet. Acquisitions of 30x30x40 μm^3 were performed during 73 min with a standard 3D gradient-echo RF-spoiled sequence, a TR/TE of 124/12 ms and a bandwidth of 63.4 Hz/pixel before (D0), 4 (D4) and 8 (D8) days after the seeding. . Magnetically labeled cells were identified as hypo-intense spots on magnitude acquisitions and their magnetic effect was quantified through the measure of the signal variance normalized by the signal mean (SV) on modulus images. The characterization of microstructural anisotropy of cell migration within scaffold was enlightened by measuring the Mean Intercept Length (MIL) number[4] in a sphere selected in the acquisition data. We defined as MIL_{min} and MIL_{max} respectively the minimum and maximum MIL number both linked to different orientation. In parallel, fluorescein isothiocyanate–labeled scaffolds were studied by confocal imaging and cells were evidenced with tetramethylrhodamine-phalloidin staining actin filaments.

Results

A 30x30x40 μm^3 resolution combined with a mean Signal to Noise Ratio (SNR) of 15 ± 3.6 was able to depict both the porous organization (Fig 1. a) of the scaffolds and the labeled cells within (Fig. 1 b, c, e and f). The signal heterogeneity linked to the presence of the labeled cells appeared clearly through the SV values which varied significantly from 1 to 7 as a function of culture time (**Error! Reference source not found.**g). The anisotropy represented by MIL_{min}/MIL_{max} decreased by 21 % between Day 0 and Day 8. All these results were confirmed by confocal imaging of fluorescein isothiocyanate-labeled scaffold (Fig. 1 d, e and f).

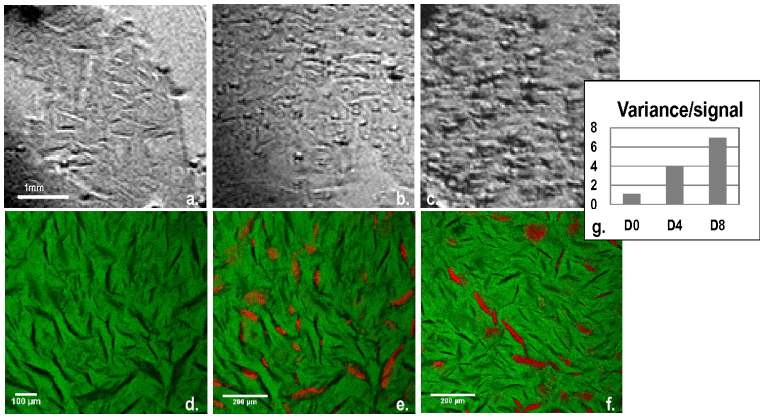

Figure 1– µMRI (top) and confocal microscopy imaging (bottom) of porous scaffolds without cells-D0 (a, d), at day 4-D4 (b, e) and at day 8-D8 (c, f), g. SV value as a function of time.

Discussion/Conclusion

High resolution 1.5 T MRI combining with a HTS surface coil with efficient medium and cellular contrast agents was proven to be an effective approach for non-invasive 3D visualization of tissue-engineered constructs. This was already shown for the porous scaffold depiction[5], and now for the follow-up of cell migration within it. The labeled cell presence was quantified, SV increased by 600 % between Day 0 and Day 8, within all the structure. Their spatial distribution, along the privileged orientation of the pores was assessed through the micro structural anisotropy increase of 21%. According to these results, cells spatial distribution, a major parameter in tissue reconstruction, is easily monitored through the complex microstructure of scaffold. MRI appears as a powerful evaluation technique to study cell seeding and migration as well as to assess clinical outcomes after in vivo implantation.

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