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SPIO-enhanced MRI as a nondestructive in vivo method to assess vascularization of 3D Degrapol® scaffolds planted on the chorioallantoic membrane of the chick embryo in ovo

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SPIO-enhanced MRI as a nondestructive *in vivo* method to assess vascularization of 3D Degrapol® scaffolds planted on the Chorioallantoic membrane of the chick embryo *in ovo*

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Abstract

MRI has recently been presented as a nondestructive *in vivo* readout to report perfusion capacity in biomaterials planted on the CAM in the living chick embryo *in ovo*. Perfusion capacity was assessed through changes in T1 relaxation pre- and post-injection of a paramagnetic contrast agent, Gd-DOTA (Dotarem®). Hence local contrast agent concentration was dependent on perfusion, vascular permeability, and extravascular compartment size. In the present study we, therefore, explore intravascular SPIO particles of the FeraSpin® series to deliver a more direct measure of vascularization in a 3D polymer DegraPol® scaffold. Furthermore, we present contrast enhancement upon SPIOs of different particle size, namely FeraSpin® series XS, M, XXL and Endorem® for comparison, and hence different efficiency on T1 and T2, and study respective dose-effects. No signal change was observed within the egg yolk, consistent with the SPIO remaining in the vasculature. Consequently, T1 positive signal enhancement (reduction in T1) and T2 negative contrast (reduction in T2) were observed only in the vasculature and hence were restricted mainly to the surface of the CAM at the interface to the biomaterial. Furthermore, the effect upon T2 appears stronger than in T1 with all SPIOs investigated and at blood concentrations between 0.46 mM to 4.65 mM. Comparison of different concentrations shows larger T1 enhancement at the highest dose, as expected. Vessel structures in and around the scaffold as seen in MRI were corroborated by histology. Different particle sizes show reduced T1 effect with larger particles, yet the effect on T2 was less apparent. In sum, SPIO-enhanced MRI provides measures for vascularization nondestructively in biomaterials connected to the CAM, based on intravascular contrast enhancement in T1 and T2, *in ovo* in the living chick embryo. Small SPIOs provide the best efficiency for that purpose, and contrast enhancement is most prominent in T2.

Introduction

Tissue-engineered biomaterials in regenerative medicine provide a matrix for cells to attach and proliferate, stimulate angiogenesis and sustain long-term function and survival of the implant. The chorioallantoic membrane (CAM) of the chick embryo is a model for studying vascularization *in vivo* [1]. As a consequence, effects of the biomaterial's volume, pore size and pore interconnectivity on the vascularization capacity can be studied [2].

Recently, an MRI method was presented as a nondestructive *in vivo* readout of perfusion capacity in biomaterials planted on the CAM in the living chick embryo *in ovo* [3]. Perfusion capacity was assessed in various scaffold materials through changes in T1 relaxation pre- and post-injection of a paramagnetic contrast agent, Gd-DOTA (Dotarem®, Guerbet S.A.). Hence, local contrast agent concentration was dependent not only on perfusion but also on vascular permeability and extravascular compartment size, as Gd-DOTA diffuses into the interstitial space, particularly in "leaky" vessels. This is a different contrast compared to what is seen with blood pool agents staying in the vasculature. In the present study, intravascular SPIO particles of the FeraSpin® series (Viscover, Miltenyi Biotec, Germany) are therefore explored to deliver a more direct measure of vascu-

larization/ vessel architecture in 3D DegraPol® scaffolds (ab medica, Italy). We present contrast enhancement upon SPIOs of different size, namely FeraSpin® series XS, M, XXL and Endorem® (Guerbet S.A.) for comparison, and hence different efficiency on T1 and T2 and investigate respective dose effects.

Objective

To assess contrast enhancement in a 3D polymer (DegraPol®) scaffold upon injection of SPIOs as intravascular contrast agents in the CAM assay. SPIO particles of different size were used and dosages varied.

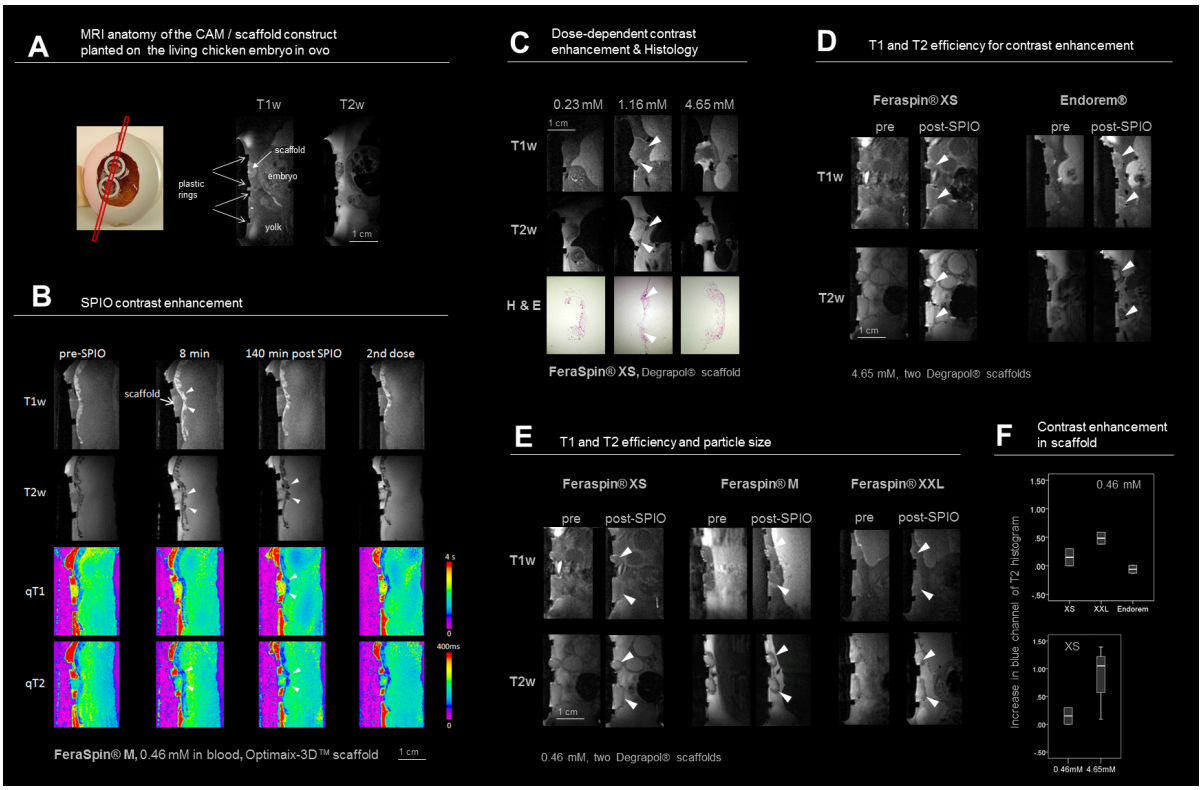

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Figure Legend

Figure 1. SPIO-enhanced MRI to assess vascularization of 3D Degrapol scaffolds planted on the chorioallantoic membrance of the chick embryo in ovo.

A MRI anatomy of the CAM/scaffold construct planted on the chicken embryo *in ovo*. Left: Picture was taken from the opened egg showing the plastic rings with the scaffolds inside as they were planted on the CAM surface. Red line denotes the sagittal slice imaged with MRI covering the two scaffolds. Right: T1w and T2w MR images taken from the sagittal slice. Denoted are the structures as visible with the MRI such as parts of the chicken embryo, egg yolk, plastic rings, scaffold.

B SPIO contrast enhancement. *In ovo* T1w (TR 800 ms, TE 9.3 ms) and T2w (TR 4.5 s, TE 83.7 ms) MRI images and corresponding color-coded quantitative T1 and T2 maps acquired in a sagittal slice through an Optimaix-3D™ scaffold. Images shown zoomed into the plastic ring containing the scaffold. Vessel structures on the CAM are indicated by arrowheads and show contrast enhancement in T1 (positive, signal increase) and T2 (negative, signal reduction) upon FeraSpin® M administration at a dose corresponding to 0.46 mM concentration in blood upon injection. Left to right: pre-, 8 and 140 min post-injection and after a second dose.

C Dose-dependent SPIO contrast enhancement and comparative histology. *In ovo* T1w- and T2w MR images of a DegraPol® scaffold and surrounding vessels at different SPIO

concentrations (FeraSpin® XS). Vessel structures are indicated by arrowheads and corroborated by H & E histology. Enhancement in T1 and T2 is highest at the highest dose.
D T1 and T2 efficiency for contrast enhancement. T1w and T2w MR images of two Degrapol® scaffolds, planted on the CAMs of two eggs, pre- and post-injection of Feraspin® XS (left) and Endorem (right) SPIO particles, respectively, showing qualitative T1 and T2 contrast enhancement upon the two different contrast agents. Contrast enhancement is more prominent in T2 than T1 with both SPIOs at 4.65 mM concentration. Larger vessels display largest T2 effect (signal reduction), outweighing T1 enhancement and hence appear dark in T1w images ("T2-shine-through").
E T1 and T2 efficiency with particle size. Contrast enhancement in T1w and T2w MR images of two Degrapol® scaffolds, planted on the CAMs of three eggs, upon injection of different sizes of particles from the same Feraspin® SPIO particle family. Feraspin® XS 10-20 nm, M 30-40 nm, and XXL 60-70 nm hydrodynamic diameter, respectively, show reduced T1 effect with larger particle size, as expected; the effect on T2 is not apparent at 0.46 mM concentration.
F Analysis of contrast enhancement within the scaffold. Histograms of color-coded quantitative T1 and T2 maps obtained pre- and post-injection were analyzed within the scaffold. Increase in the blue channel– corresponding to decrease in T1 and T2 values– is shown as a measure of contrast enhancement and depicted with respect to SPIO particle size and SPIO concentration of the contrast agent. Only data for T2 are shown which revealed an appreciable trend.

Results & Discussion

MR images were obtained from one sagittal slice positioned through the scaffold(s) planted on the CAM of the chicken embryos (Figure 1A). A medetomidine anesthesia protocol offered proper sedation of the chick embryo throughout the MRI acquisition and the scaffold was clearly and reproducibly depicted.
No signal change was observed within the egg yolk, consistent with the SPIO remaining in the vasculature. Consequently, T1 positive signal enhancement (reduction in T1) and T2 negative contrast (reduction in T2) were observed, consistently in all samples, in the vasculature and hence were restricted mainly to the surface of the CAM at the interface to the scaffold material where vessel density is highest (Figure 1B). The effect upon T2 appears stronger than in T1 with FeraSpin® M particles at 0.46 mM concentration in the Optimaix-3D™ scaffold.
Remarkably, no change in contrast was observed inside the scaffold, which might relate to partial volume effects between the small vascular compartment as compared to the scaffold structure, but also to our observation that the slightly viscous contrast agent did not distribute easily but rather slowly within the finer segments of the vasculature, like the vessels penetrating the scaffold. Consistent with this notion are the viscous quality of the FeraSpin® SPIO preparation and the observation that T1 and T2 contrast enhancement was more prominently seen at 140 min than 8 min after injection of the SPIO. Dose escalation from a second SPIO injection did not result in notable further contrast enhancement, possibly for the same reason.
Qualitative comparison of different FeraSpin® XS concentrations shows larger T1 enhancement at the highest dose, as expected (Figure 1C). For comparison, doses typically suggested for use in mice are in a similar range, such as 0.66 mM (mouse example from manufacturer administering 0.1 mL of 10 mM FeraSpin®) and about 9 mM used for q-mapping [4], administering monocrystalline iron oxide nanoparticles MION at 30 mg/kg body weight. Vessel structures as seen in MRI are corroborated by histology.
T1 and T2 efficiency for contrast enhancement is dependent on relaxivity of the SPIO particles and hence on particle size, as well as on clearance of the contrast agent from the blood compartment (blood circulation time itself dependent on particle size). T1 and T2 contrast enhancement with FeraSpin XS® and Endorem® in Degrapol® scaffolds is depicted in figure 1D for comparison. At the same contrast SPIO concentration (4.65 mM) contrast enhancement appears more prominent in T2 than in T1. Moreover, larger vessels present strong T2 effect (signal reduction) outweighing T1 enhancement (signal increase) and hence appear dark in T1w images due to these competing effects ("T2-shine-through").

T1 and T2 efficiency of different particle sizes of the FeraSpin® SPIO particle family are shown in figure 1E. Feraspin® particles with XS 10–20 nm, M 30–40 nm, and XXL 60–70 nm hydrodynamic diameter, respectively, show reduced T1 effect with larger particle size, as expected; effect on T2 (larger effect/signal reduction with increasing particle size) is not apparent at 0.46 mM concentration.
Quantitative analysis of contrast enhancement within the whole scaffold revealed the same trends as observed qualitatively in the MR images (Fig. 1F).

Conclusions

Our data demonstrate that SPIO-enhanced MRI is feasible in the chick embryo in the CAM assay. SPIO-enhanced MRI provides measures for vascularization nondestructively in biomaterials connected to the CAM, based on intravascular contrast enhancement in T1 and T2, *in ovo* in the living chick embryo. Small SPIOs provide the best efficiency for that purpose, and contrast enhancement is most prominent in T2.

Limitations

We compared MR images *qualitatively* for contrast enhancement and to the corresponding histological sections. In our MR assessments, the intravascular contrast was most apparent in large vessels directly at the interface between the CAM and the biomaterial planted on it. We are now working on a *quantitative* assessment of this region in order to quantify the effect of different SPIO particle sizes and concentrations of SPIOs applied. A thorough detailed quantitative analysis, however, requires:
-more samples per condition (SPIO particle type, concentration) and,
-to tackle dependence on ROI selection, as it varies greatly with positioning within the scaffold (especially when touching the more vascularized region towards the interface to the CAM), the size of the selected ROI (due to partial voluming between vessels and scaffold material), and the effect of single big vessels. Alternatively, a well-defined control region within the yolk may be impeded by the motion of the chick embryo between pre/post-injection MRI sessions.
Yet, a *quantitative* assessment of the scaffold region with the purpose to quantify the effect of different SPIO particle sizes and concentrations of SPIOs was conducted using a histogram analysis of color-coded T1 and T2 maps, which allowed to assess the more subtle signal changes within the scaffold region. This analysis confirmed contrast enhancement (reflected in the increase in the blue distribution, which in turn corresponds to decrease in T1 and T2 relaxation times within the scaffold, upon contrast agent administration) and reproduced the trends observed qualitatively, as described above, for the interface region between CAM and scaffold (Figure 1F): Larger effects were observed at higher contrast agent concentrations, and contrast enhancement was more prominent in T2 than T1. Furthermore, increased effect on T2 was expected, and observed, with increased particle size (in the FeraSpin® series, not Endorem, however) in the scaffold region, while effect on T1 was expected to be reduced but was not obvious in the scaffold region, arguably due to underlying T2-effects (T2 shine-through).
Full thorough quantitative analysis of contrast enhancement, in particular *within* the scaffold, requires more samples per condition and was replaced by a semi-quantitative histogram-based analysis on our preliminary data, which is supposedly more sensitive towards smaller signal changes within the scaffold. Results from this analysis reproduced the qualitative observations/trends seen in the (more obvious) interface region between the CAM and scaffold.

Additional Information

Methods and Supplementary Material

Please see https://sciencematters.io/articles/201710000003.

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Ethics Statement

Not applicable.

Citations

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