Identifying Contributions from Scaffolds, Cells and Extracellular Matrix in MRI of Polymer-Hydrogel-based Engineered Cartilage

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Statement of Purpose

Development of non-invasive imaging of engineered tissues is of utmost important for tracking the progress and predicting the success and failure of tissue engineering approaches. MRI is leading non-invasive characterization technique suitable for follow-through in preclinical and clinical stages. Current MRI characterization methods for engineered cartilage tissues are based on correlation of water MR parameters, for example the relaxation time T_2, with extracellular matrix components, proteoglycans and collagen [1-2]. This method is based on such correlation found in natural cartilage tissue [1]. However, it ignores the interaction of water protons with cells that includes both intracellular and extracellular spaces, and biocompatible scaffolds. These are often the dominating contributions in water MR parameters of growing engineered tissues.

In the current study, we present preliminary MRI data for chondrogenic differentiation of human bone marrow derived stem cells seeded onto the specially designed "polymer-hydrogel" osteochondral matrices to separate out various contributions in water T_2 relaxation time. The scaffold system is uniquely designed to best support osteochondral defect repair and regeneration. The MRI characterization of these chondrogenic scaffolds was performed for scaffold only, scaffold with cells and ECM, and scaffold with ECM without cells. We identified the contribution of ECM and cells in water T_2 that proves to be much larger than previously shown by other groups.

Materials and Methods

Scaffold Preparation: We designed "Polymer-Hydrogel" Scaffold with tuned gradient properties [3-4]. As a first step, bone marrow derived human mesenchymal stem cells (BM-hMSCs) were combined with puramatrix hydrogel. These human bone marrow stromal cells (500 K/scaffold) embedded in hydrogel were added to the pores of the gradient matrix and the cell-seeded matrices were cultured in chondrogenic media for cellular differentiation and matrix formation [5]. The scaffolds were 4 mm in diameter and 8 mm in height. The production of proteoglycans and collagen was confirmed by biochemical analysis.

MRI experiments: All MRI experiments were performed in chondrogenic growth media to preserve the natural environment of engineered tissues. We used Bruker Avance DRX 11.7 T (500.17 MHz) Spectrometer with 5 mm RF coil, FOV = 11 mm x 11 mm, In-plane resolution = 0.086 mm x 0.086 mm, slice thickness = 0.5 mm, matrix size = 128 x 128, number of slices = 7. After performing MRI on engineered cartilage that contained cells, the samples were fixed in formalin. The samples were washed three times in media on the following day before performing MRI measurements again.

Results: The T_2 values for cells and ECM were calculated using the following equation:

$$\frac{1}{T_{2(cells,scaffold,ECM)}} = \frac{1}{T_{2,scaffold}} + \frac{1}{T_{2,cells}} + \frac{1}{T_{2,ECM}} \qquad (1)$$

Table 1 presents the measured T_2 values in the first three columns with specific data shown in Figure 1 (highlighted yellow) and calculated T_2 values for water-cells and water-ECM independently (boxed red). It is clear from the table that the contribution of water-scaffold interaction in T_2 is the most dominating contribution for these tissues. It would hinder the observation of growing ECM and proliferating and differentiating cells if not accounted for. These calculated values indicate that cells and newly deposited ECM have mobile fractions, thus have higher T_2 values. The calculated T_2 values are much larger than published reports. Further work is underway to validate our results.

Table 1: Contribution of ECM, cells and scaffold in T_2 MRI	T_2 (Cells +ECM+ Scaffold) (ms)	T_2 (ECM+ Scaffold) (ms)	T_2 (Scaffold) (ms)	T_2 (ECM) (ms)	T_2 (Cells) (ms)
Day 7	88.04 [B]	89.64 [D]	96.88 [A]	1251.8	894.2
Day 19	89.33	101.91	96.88 [A]	880.9	608.6
Day 26	90.1 [C]	113.7 [E]	96.88 [A]	1016.2	619.8

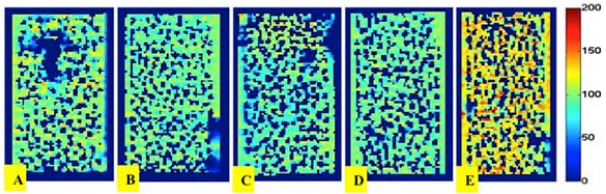

Figure 1: T_2 maps for (A) scaffold (B) engineered cartilage (scaffold + cells + ECM) at day 7 (C) engineered cartilage at day 26 (D) engineered cartilage-without-cells (ECM + scaffold) at day 7 (E) engineered cartilage-without-cells at day 26. Without-cells measurements were taken on fixed samples. The color-bar is in ms.

Conclusions

Through this study for the first time, we applied MR parametric imaging tool to monitor the progress of cartilage tissue regeneration in a specially designed and advanced osteochondral scaffold seeded with human bone marrow derived stem cells. This study shows that MR parametric imaging is a sensitive tool to study tissue engineered cartilage. The long-term goal of this study is to develop a non-invasive method to monitor the progress of tissue regeneration in an osteochondral graft system designed for osteochondral defect repair and regeneration.

References

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