T_2 tracks microstructural changes in implantable, iron oxide-doped hyaluronic acid/collagen hydrogels for preclinical and regenerative medicine.

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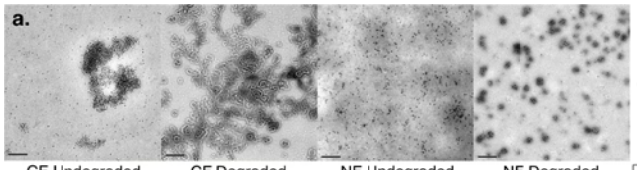
Introduction. Hyaluronic Acid (HA) is an essential extracellular matrix (ECM) component that mediates cellular signaling, wound healing, cell morphology, and matrix organization (1); HA degradation products induce angiogenesis (2) and influence neocartilage formation by mesenchymal stem cells (3). In situ crosslinking formulations are used preclinically to increase cell survival and are being developed for clinical use in conjunction with cell therapy (1). Real-time degradation measurements potentiate monitoring of the entire course of cell therapy and optimization of structural properties during that period. These measurements also facilitate research of cell-ECM interactions. A technique to monitor changes in macromolecular structure has been developed (4,5) in which functionalized superparamagnetic nanoparticles are bound to structural macromolecules of the ECM. Cells interacting with the labeled ECM induce a change in nanoparticle aggregation state, which is detected as a change in T_2. Here we show that by doping hydrogels with functionalized iron-oxide nanoparticles, MRI can be used to precisely measure HA degradation. T_2 changes in the hydrogel scaffold during degradation are consistent with scanning electron microscopy (SEM) images of hydrogel structure and transmission electron microscopy (TEM) images of nanoparticles embedded in those hydrogels. This work enables measurements of hydrogel structure in real time, in vivo.

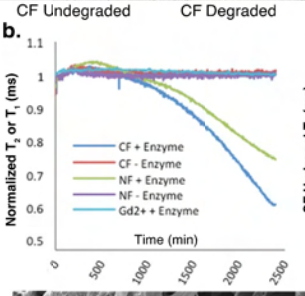
Methods. A biocompatible hydrogel of collagen and heparinized HA (hHA, HyStem-HP, Glycosan Inc.), was doped with native ferritin (NF) or cationized ferritin (CF) nanoparticles, as described in (6). The gel was then degraded either enzymatically with 5 ul/ml hyaluronidase/collagenase (1000/3000 U/ml, StemCell Technologies) or plated with C6 glioma cells at 37°C, 5% CO_2, for 7 days; control gels received no enzyme or cells during the incubation period. As a control, 5 uM Magnevist was also embedded in a degrading hydrogel and measured with a repeated inversion recovery sequence tau=5-2000 ms/TR=15 s. T_2 measurements were made in a 1.5T Bruker mq60 relaxometer using a Carr-Purcell-Meiboom-Gill (CPMG) sequence repeated every 140 s. Cell T_2-maps were generated on a Bruker 7T BioSpec scanner using a multi-shot multi-echo (MSME) sequence TE/TR = 11/800. For TEM, 10 ul of sample was wicked across Formvar-coated copper mesh and images were taken on a Phillips CM12 TEM at 80 kV. For SEM, samples were flash-frozen in liquid nitrogen, stored at -80°C, lyophilized, sputter-coated for 300 s with gold, and imaged using a FEI XL30 ESEM at 15 kV; 40 pores were measured manually, and a one-way ANoVA with a Tukey-Kramer correction was applied to adjacent time points with alpha=0.05. In vivo T_2-maps were generated from images taken with a MSME sequence TE/TR = 11/4622. The CF or NF concentration for all samples was 1 uM.

Results. Figure 1 shows HA hydrogels with embedded contrast agents degraded enzymatically. As shown in (1a), the aggregation state of the CF or NF in the hydrogel changed during degradation; CF clusters changed from of ~0.47 um to ~0.06 um and NF changed from unclustered to ~0.05 um clusters. These changes in clustering accompanied a drop in T_2 as shown in (1b). No change occurred without enzyme. No signal change occurred when a T_1-agent was used instead of ferritin; a T_1 agent should change T_1 if there were altered proton density or exchange inside the hydrogel. SEM of freeze-dried hydrogels showed microstructural changes in hydrogels; pore diameter increased from 7.3±3.7 um to 19.9±9.5 um during the first 190 minutes of degradation (1d,1e). A similar change was observed in hydrogels degraded by cells (Fig. 2, 2b). When cells were grown in doped hydrogels, T_2 was 352±81 ms; in controls it was 206±87 (2a). In TEM images of cell-degraded hydrogels, CF formed clusters ~1 um in diameter; without cells CF formed ~0.025 um clusters (2c). Finally, hydrogels were injected subcutaneously and detected in vivo with MRI (2d).

Conclusion. This work demonstrates that changing the aggregation state of superparamagnetic nanoparticles causes changes in T_2 in a biocompatible hydrogel, which can be used to report on the microstructure of the hydrogel. T_2 is longer in hydrogels with large (>300 nm) clusters of particles or unclustered particles than in hydrogels with clusters under 100 nm. Enzymatically degrading hydrogels labeled with NF, which will not bind to heparin on the HA, or CF, which binds to and clusters around heparin, shortens T_2 as clusters approach 50 nm. (1b) shows that this transition is more sensitive; at least a 40% signal drop occurred when CF was embedded as opposed to NF. In contrast to the enzymatically degraded hydrogels, gels degraded by cells exhibited an increase in T_2. We observed large clusters of CF in these degraded samples (2c). T_2 changes due to water diffusion through magnetic perturbations were previously described (7,8). The changes in hydrogel structure, as opposed to ferritin aggregation state, can were visualized by SEM as in (1d,1e). This work makes it possible to precisely measure the polymerization state of an implanted biocompatible hydrogel with MRI.

References. 1. Prestwich G. J Controlled Release. 155 (2011) 193-199. 2. West DC et al. Science. 228 (1985). 3. Chung C et al. Biomaterials. 30 (2009) 4287–4296. 4. Bennett KM et al. Biophys J Vol. 95 (2008) 342-351. 5. Colomb J et al. Mag Reson Med. 64 (2010) 1792-1799. 6. Danon et al. J Ultrastructure. 38 (1972) 500-510. 7. Brooks. Mag Reson Med. 47:2 (2002) 388-391. 8. Yablonskiy and Haacke. Mag Reson Med. 32:6 (1994) 749-763.





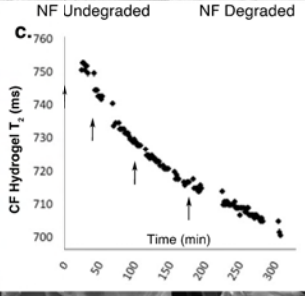
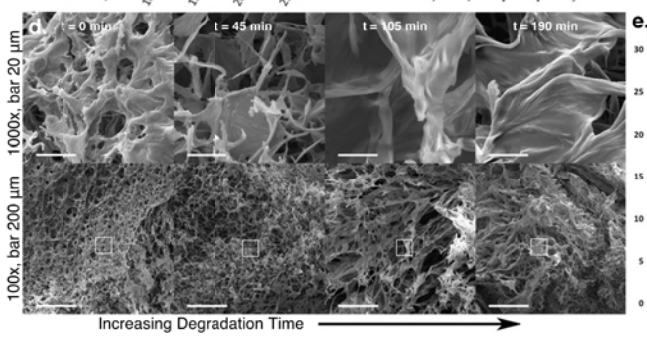


Fig 1. Images corresponding to enzymatic degradation of hHA hydrogels. (a) TEM images of hHA hydrogels with embedded CF of NF particles, before or after enzymatic degradation; black dots are ferritin cores, 66x magnification, 0.1 µm scale bars. (b) T_2 measurements of hHA hydrogels with embedded contrast agents, enzymatically degraded with hyaluronidase/collagenase (5/15 U/ml) measured every 140 s with a CPMG sequence TE/TR = 5/10000 ms, normalized to value at t=100 min. (c) hHA hydrogel with embedded CF, degraded with hyaluronidase/collagenase (5/15 U/ml) measured every 140 s with a CPMG sequence TE/TR = 1/10000 ms. (d) SEM images of gold-coated hHA hydrogel, times corresponding to arrows in (c), with mean pore diameter (e), 100x and 1000x images with 200 µm and 20 µm scale bars.



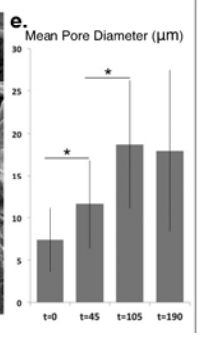


Fig 2. Images corresponding to cell degradation of hHA hydrogels. (a) MRI images (below) and T_2 measurements (above) of hHA hydrogels, calculated from images taken with a MSME sequence TE/TR = 11/4,814 ms, scale 2-178 ms. (b) SEM images of gold-coated hHA hydrogels from (a),embedded with CF and cells or no cells, incubated in DMEM for 7 days. (c) TEM of hydrogels from (a) taken at 140kx and 66kx, scale bars 50 nm or 100 nm; black dots are ferritin cores. (d) is a T_2-map of hHA hydrogels embedded with CF (right) or NF (left), taken with MSME sequence TE/TR = 11/4622 ms, scale 206-407 ms.