Towards Translation of MRI-Detectable Hydrogels for Cell Therapy and Tissue Regeneration

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Introduction. Nanostructured hydrogels have been developed as scaffolds for cell delivery in vivo (1). Hydrogels provide structure for cells to grow in 3D and guide them in tissue regeneration. One challenge in hydrogel development is the need to non-invasively monitor macromolecular structural changes as cells rearrange the gel after implantation. A technique to noninvasively detect changes in hydrogel macromolecular structure with MRI has recently been reported (2,3) based on imbedding functionalized magnetic nanoparticles in the hydrogel before the gel is formed. The nanoparticles bind to specific macromolecules in the gel, and aggregate around them. The particle aggregation state changes when the gel is degraded, causing a change in the MRI signal through a change in T_2 relaxation time. Controlled aggregation is an effective way to detect macromolecular polymerization state (2,3), which thus allows noninvasive monitoring of the molecular structure of the hydrogel. Here we develop this technology in a biocompatible hydrogel, report T_2 changes over time in vitro consistent with cellular rearrangement of the matrix, demonstrate that this technique can be used to detect cell proliferation in the hydrogel, and show that contrasted gels can be visualized in vivo. Unlike previous work with synthetic fibers and Matrigel (3), the components of this hydrogel are all FDA approved, and thus will be more readily translated for human use.

Methods. We created a biocompatible MRI reporter gel using HyStem-HP (Glycosan Inc.), a hydrogel made primarily of hyaluronic acid (HA) and collagen, crosslinked with bifunctional poly(ethylene glycol) diacrylate. Negatively charged thiol-modified heparin is bound to the HA, to which we attached 1 µM cationic ferritin nanoparticles (CF). As proof of concept, 1.25 ml hydrogel samples were degraded using 30 µl 10x Hyaluronidase/Collagenase (Stemcell Technologies, cat. 07912); T_2 was measured every 140 seconds on a Brucker 60 MHz spectrometer, using a CPMG pulse sequence TE/TR = 20/2000 ms. For in vitro measurements of cells proliferating in gels, wells containing 500 µl HyStem HP with 1 µM CF were plated ± rat c6 glioblastoma cells, and cell growth was confirmed by microscopy; cells were imaged 8 days later on 7T Bruker MRI system with a spin echo sequence, TE/TR = 20/633. For in vivo testing, .8 ml gels were then injected subcutaneously into ~250g male Sprague Dawley rats, then gradient echo images and spin echo T_2 maps were generated TE/TR = 20/422. The image in figure 1 is representative of an n=6 experiment. T_2 maps were generated using an MSME sequence with an arrayed TE (11-176 ms) and TR = 4622 ms.

Results. In vitro samples (Figure 1) showed a 33% drop in T_2 over the first 3-5 hours, before rising. In all samples, during the first minutes signal increased as the gel crosslinked, and then as CF settled out of the liquefied gel, T_2 rose 200% (to ~790 ms). This is consistent with the measured T_2 value of gels containing no contrast agent. The mechanics of degradation is varied between samples; we hypothesize this is because the gel does not degrade evenly. In vitro, rat c6 glioma cells plated in the gel caused a drop in T_2; measured values were 134 ± 27 ms and 119 ± 42 ms (cells), and 188 ± 41 ms and 157 ± 39 ms (control) ($p < .05$). In vivo measurements confirm that gels are visible, and that addition of CF causes a drop in measured T_2 from 311 ± 52 ms to 127 ± 40 ms.

Discussion. It has been shown that degradation state of nanoparticle-doped hydrogels can be measured by a change in T_2. We have demonstrated here that this phenomenon can be used to track changes in gel aggregation state caused by enzymatic degradation or by cell migration. We have further shown that gels can be detected in vivo, expecting that we will also be able to detect reduced T_2 from cells migrating in vivo. This could be used to detect the viability, migration, and division of cells implanted with the gel; or it could detect migration of cells into a gel injected without cells, which would allow for quantitative assessment of stem cell delivery or regeneration of tissue.

References. 1) Serban M et al. Prot. Cell (2008) p.3946. 2) Bennett K et al. Biophysical Journal Vol. 95 (2008) p. 342-351. 3) Colomb J et al. Magnetic Resonance in Medicine. In Press. (2010).

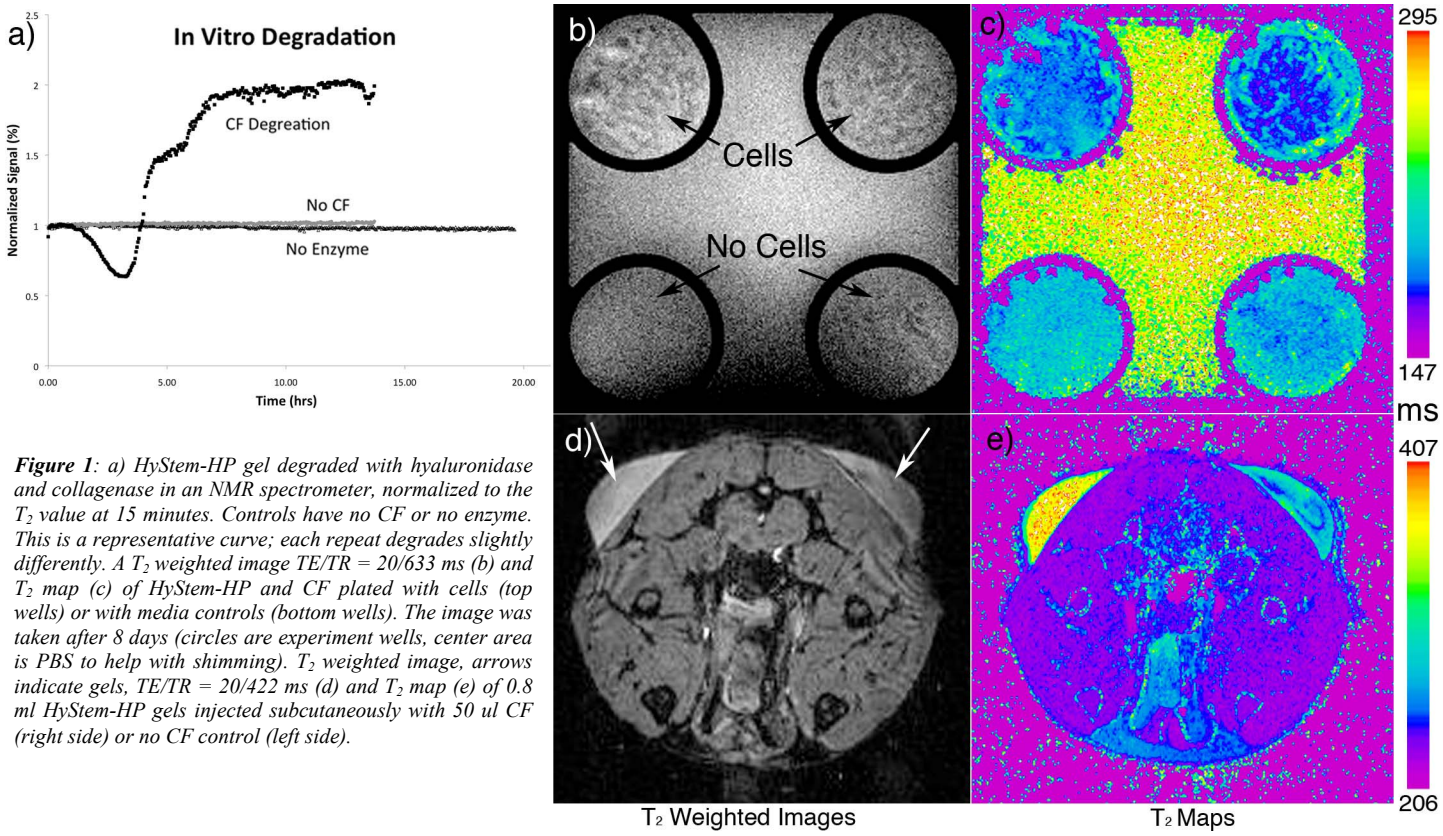

***Figure 1**: a) HyStem-HP gel degraded with hyaluronidase and collagenase in an NMR spectrometer, normalized to the T_2 value at 15 minutes. Controls have no CF or no enzyme. This is a representative curve; each repeat degrades slightly differently. A T_2 weighted image TE/TR = 20/633 ms (b) and T_2 map (c) of HyStem-HP and CF plated with cells (top wells) or with media controls (bottom wells). The image was taken after 8 days (circles are experiment wells, center area is PBS to help with shimming). T_2 weighted image, arrows indicate gels, TE/TR = 20/422 ms (d) and T_2 map (e) of 0.8 ml HyStem-HP gels injected subcutaneously with 50 ul CF (right side) or no CF control (left side).*