Self-degrading, MRI-detectable hydrogels with picomolar target sensitivity

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Introduction: Nanostructured hydrogels have been developed as synthetic tissues and scaffolds for cell and drug delivery, and as guides for tissue regeneration. A fundamental problem in the development of synthetic hydrogels is that implanted gel structure is difficult to monitor noninvasively. MRI has been used to detect hydrogel structural alterations for radiation dosimetry[1]. Other structures have been detected by embedding functionalized contrast agents[2]. Controlled aggregation has been used to detect gel shrinking and swelling[3], to create enzyme-sensitive agents[4, 5, 6], and to detect actin polymerization in vitro[7]. Here we demonstrate that the aggregation of cationic magnetic nanoparticles, attached to specific macromolecules in biological and synthetic hydrogels, can be controlled to detect changes in gel macromolecular structure with MRI. We further caused the macroscopic "reporter gels" to self-degrade in the presence of a target enzyme and controlled the gel sensitivity by embedding a molecular zymogen (inactive enzyme) cascade, to cause the gels to self-degrade in response to pM target concentrations of target. This work is a first step to MRI detectable synthetic hydrogels and implantable, MRI detectable biosensors.

Methods: We created both biological and synthetic reporter hydrogels. We added cationic nanoparticles, which bound to anionic groups in the gel, and caused them to cluster and uncluster with gel enzymatic degradation. To create biological reporter gels, Matrigel (BD Biosciences) was doped with cationic nor native, unfunctionalized ferritin (CF or NF, Sigma Aldrich), which are superparamagnetic. To create synthetic fiber reporter gels (SFRGs), we electrospun dextran-poly(acrylic acid) (dex-PAA) hydrogel microfibers and electrostatically bound them to CF nanoparticles. Fibers were conjugated to FITC for fluorescence. A second class of SFRG was created by conjugating the fibers to the CF with a serine-protease cleavable peptide spacer. These were digested with either 5-10 μM trypsin or 1-20 nM dextranase. To create a zymogen cascade, we embedded trypsinogen and triggered its activation to trypsin with 0-10.96μM EK enterokinase (EK). Gels were digested at 37°C, and we measured T_2 during digestion with a Carr-Purcell Meiboom Gill (CPMG) sequence, TE/TR = 10/10000 ms, on a Bruker Minispec mq60 relaxometer. We measured T_2 in the gels *in vivo* by imaging a subcutaneous implant of 300 μl in a rat flank, and imaged with CPMG (TE/TR = 4/3000 ms) after 2-6 days on a Bruker Biospin 7T scanner. We used stereo epifluorescence (Leica MZFLIII) to confirm fiber structure in gels. Transmission and scanning electron microscopy (after lyophilizing and gold sputtering) were performed on a Philips CM12S (TEM) and a XL-30 ESEM-FEG (SEM).

Results and Conclusions: Degradation of synthetic fiber RGs with dextranase caused the fibers to shorten and cluster, as shown by FITC-labeled, nanoparticle-doped, SFRGs (Figs 1a and 1d) and in SEM images (Fig.1b, 1e). In TEM (Fig. 1c, 1f), the degradation of the dextran/PAA fibers causes an increase in fiber thickness. Bound nanoparticles were densely distributed on the fiber surface (Fig 1g). As the SFRG was degraded by dextranase, the T_2 of the SFRG increased by 125% (Fig 1h). SFRGs with trypsin cleavable sidechains covalently bound to nanoparticles had a 26% decrease in T_2 when digested with trypsin (not shown). In zymogen-enhanced gels, the onset of degradation in response to 4 pM target EK was controlled by the concentration of zymogen (Fig. 2). Subcutaneously implanted reporter gels had 10% higher T_2 after 6 days, as detected with *in vivo* MRI (Fig 3). We conclude that gel macromolecular polymerization state can be detected with MRI by controlled aggregation, and that these gels can be finely tuned using molecular amplification to detect ~pM concentrations of target enzymes. This work raises the possibility of self-digesting, MRI-detectable implants and scaffolds for drug delivery, or for implantable hydrogel biosensors.

References: (1) Gore, JC et al. Magn Reson Med 9, 325-332 (1989). (2) Bull, SR, Guler, MO, Bras, RE, Meade, TJ, Stupp, SI. Nano Lett 5, 1-4 (2005). (3) Shachar, F, Lauterbur, PC. Nature 363, 334 - 336 (1993). (4) Atanasijevic, T, Shusteff, M, Fam, P, Jasanoff, A. Proc Natl Acad Sci U S A 103, 14707-14712 (2006). (5) Wunderbaldinger, P, Josephson, L, Weissleder, R. Acad Radiol/9 Suppl 2, S304-306 (2002). (6) Zhao, M, Josephson, L, Tang, Y, Weissleder, R. Angew Chem Int Ed Engl 42, 1375-1378 (2003). (7) Bennett, KM et. al. Biophys J. 95, 342-351 (2008).

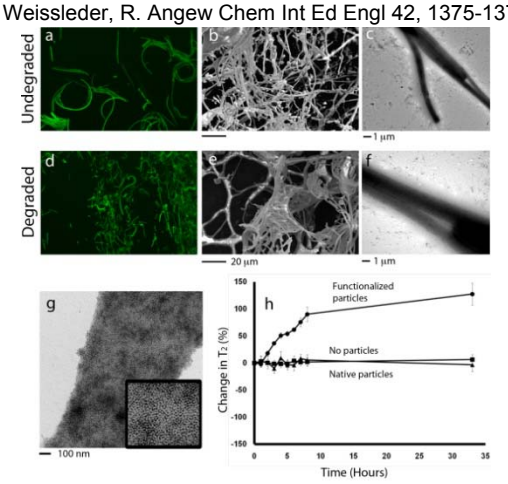

Figure 1: Images and MR of electrospun Dex-PAA as a synthetic reporter gel. (a) Fluorescence of FITC-labeled fibers before degradation by 10 nanomolar dextranase, (b) SEM of fibers before degradation with dextranase, (c) TEM of a fibers with cationic nanoparticles attached to the surface before digestion with dextranase, (d) Fluorescence of FITC-labeled fibers after degradation with dextranase, (e) SEM of fibers after degradation with dextranase, (f-g) TEM of labeled fibers after degradation with dextranase, with bound nanoparticles shown as electron-dense spots in the magnified frame. (h) T_2 increases rapidly when the fibers are degraded by dextranase.

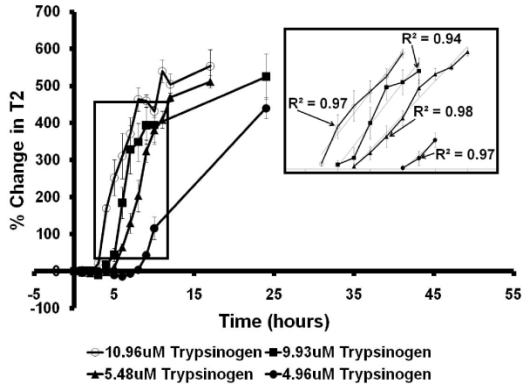

Figure 2: Reporter gel degradation can be controlled with a zymogen cascade. MR relaxation show that the onset time of reporter gel degradation by 4 pM enterokinase can be controlled by changing the concentration of trypsinogen zymogen.

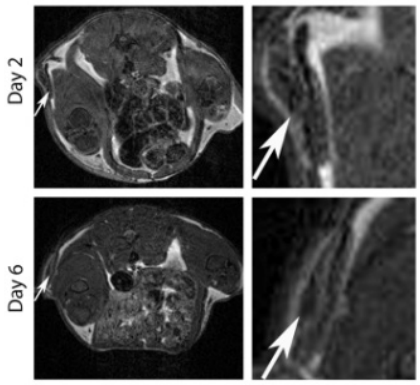

Figure 3: *In vivo* T_2-weighted images of subcutaneous biological reporter gel injected into a rat flank show a 10% increase in T_2 between day 2 and day 6 after implantation. Arrows show the subcutaneous gel. T_2 increased from 50 ms to 55 ms in the implant, suggesting *in vivo* degradation of the gel. Native gels (not shown) had no similar T2 change.