# MRI reporter gels to detect enzymes and cells in vivo

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**Introduction:** The extracellular matrix (ECM) is a vital component of the interstitial space which provides a structural scaffold to support cell growth and motility, and is a reservoir of cytokines, growth factors, and cell adhesion molecules. Cells create and destroy the ECM, and the structure of the ECM depends on cellular activity. Synthetic gel scaffolds have been created to to mimic the ECM, deliver drugs, and promote tissue regeneration after injury (1). The molecular structure of gels has been designed to interact with specific cell surface proteins and enzymes (2). The goal of this work was to develop an "MRI reporter gel" which can give specific information about its molecular structure using MRI. We embedded functional iron oxide nanoparticles (ferritin) within the gel, and allowed their aggregation state to determine the gel $T_2$. The aggregation of iron oxides is known to modulate $T_2$ (3,4). Here we attached positively charged SPIOs to negatively charged heparin sulfate proteoglycans (HSPGs) in a three-dimensional ECM gel, and measured changes in MRI relaxation resulting from gel degradation with two different proteolytic and glycolytic enzymes. Because each enzyme affects the structure of the gel macromolecules differently, and therefore affect the aggregation of the iron oxides differently, the structure of the gel after degradation should thus depend on the action of the specific enzyme. Additionally, we found that the sensitivity of reporter gel degradation to a target molecule could be controlled using a cascade of self-activating zymogens (inactive proteolytic enzymes) embedded in the gel. Reporter gels are thus a versatile way to detect specific molecules in vivo with high sensitivity, and may be useful as self-reporting, biocompatible tissue scaffolds.

**Materials and Methods:** Biological reporter gels: Cationic and native horse spleen ferritin (CF and NF, Sigma) were suspended in matrigel (BD Biosciences) at 100 nM – 10 μM at $4^0$C (liquid), and mixed with 10 mM trypsin (with or without inhibitor) or heparinase I (Sigma Aldrich) in 7.4 Ph TRIS buffer. The gels were digested by incubation at $40^o$C for 24 hours. To make reporter gels degrade in the presence of target molecules, we embedded 2μM trypsinogen and 4 pM enterokinase in the gel, and digested as before. Enterokinase converts trypsinogen to trypsin, which in turn converts more trypsinogen. Magnetic Resonance: Bruker 11.7T scanner and a GE 3T Sigma Advance scanner, using a gradient-echo sequence for $T_2$* measurements. Gel relaxation measurements were made using a 20 MHz relaxometer (Bruker), using a CPMG pulse sequence with TE/TR = 10/10,000 or 60/10000 ms for trypsin and heparinase experiments. Electron Microscopy: Gels were embedded in expoxy, sectioned onto carbon grids, and imaged with a Phillips 201TEM. Light microscopy: Stereo-microscopy was done with a Leica DM IRBE microscope.

**Results and conclusions:** NF was monodisperse in the matrigel, as seen in TEM (Fig 1a), but CF aggregated (Fig 1b) in the gel due to its binding to charged gel glycosaminoglycans. This difference between NF and CF was visible as a difference in optical turbidity (1c –d). Trypsin-digested matrigel, containing CF, collapsed after 24 hours, but heparinase-digested gel was largely intact (1e-f). The collapse of CF bound to macromoleules in matrigel after trypsin caused a doubling in $T_2$, while the $T_2$ of NF-doped matrigel was unchanged (Fig 2a). Heparinase digestion did not significantly affect $T_2$ (Fig 2b), consistent with the bulk structure shown in Fig 1f. Differences between CF and NF, as well as trypsin-digested gel and gel with trypsin inhibitor were detected by MRI (Fig 2c). The degradation of CF-doped reporter gels was sensitive to extremely low (pM) concentrations of target enzyme (enterokinase), demonstrating that reporter gel degradation can be controlled. We conclude that synthetic reporter gels may be useful as of detectable, biocompatible scaffolds, and they can be designed to degrade specifically in response to a target molecule. Reporter gels may thus also be practical for observing the timed release of drugs or contrast agents upon detection of a small number of cells after they are implanted *in vivo*.

**References: (1)** Daley et al. *J Cell Sci.* 2008;121 (pt 3). (2) Park Y et al. *Tissue Engr.* 2004;10(3/4):515-522. (3) Wunderbaldinger et al. *Acad Radiol.* 2002;9(Suppl 2):S304-306. (4) Bennett et al. *Biophys J.* 2008; 95:342-351.

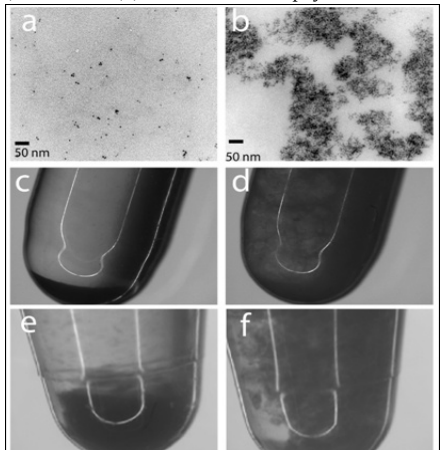


***Figure 1: Controlled aggregation of functional SPIOs in a reporter gel with degradation by specific enzymes. (a,b) TEM of native (a) and cationic (b) ferritin (NF,CF) nanoparticles in matrigel. NF is mono-disperse, while CF aggregates due to negatively charged macromolecules in the gel. (c,d) Light microscopy shows the same effect: NF in matrigel is clear (c), while CF in matrigel is turbid (d) and aggregates are visible. (e) matrigel-CF reporter gel after digestion with trypsin, and (f) after digestion with heparinase I.***

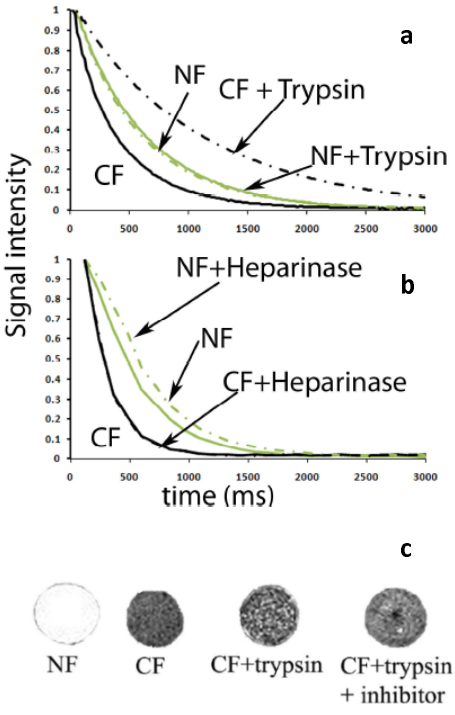


***Figure 2(left): Measured signal intensity of reporter gels during degradation using a CPMG pulse sequence. (a) Degradation with trypsin, showing increase in $T_2$ with CF, but not with NF, suspended in the gel. NF and NF+trypsin curves overlap. (b) Degradation with heparinase shows little change in $T_2$ with either CF or NF, demonstrating that the $T_2$ change is enzyme-specific. CF and CF+heparinase curves overlap. (c) Gradient-echo MRI of matrigel plugs doped with either NF or CF, with or without trypsin, showing detection of macromolecule-bound CF, and degradation of the gel***

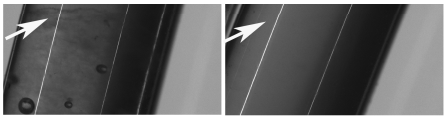

***Figure 3 Stereomicroscopy of matrigel reporter gel with 2 μM trypsinogen zymogen and either buffer (left) or 4 pM enterokinase (right). Cationic ferritin bound to gel macromolecules gives visible contrast when it is undigested ( left), but the gel is completely degraded in the presence of enterokinase (right) after 6 hours.***