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Detecting de-gelation through tissue using magnetically modulated optical nanoprobes (MagMOONs)

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ABSTRACT

Alginate gels are widely used for drug delivery and implanted devices. The rate at which these gels break down is important for controlling drug release. Since the de-gelation may be different in vivo, monitoring this process in situ is essential. However, it is challenging to monitor the gel through tissue due to optical scattering and tissue autofluorescence. Herein we describe a method to detect through tissue the chemically-induced changes in viscosity and de-gelation process of alginate gels using magnetically modulated optical nanoprobes (MagMOONs). The MagMOONs are fluorescent magnetic microspheres coated with a thin layer of opaque metal on one hemisphere. The metal layer prevents excitation and emission light from passing through one side of the MagMOONs, which creates orientation-dependent fluorescence intensity. The magnetic particles also align in an external magnetic field and give blinking signals when they rotate to follow an external modulated magnetic field. The blinking signals from these MagMOONs are distinguished from background autofluorescence and can be tracked on a single particle level in the absence of tissue, or for an ensemble average of particles blinking through tissue. When these MagMOONs are dispersed in calcium alginate gel, they become sensors for detecting gel degradation upon addition of either ammonium ion or alginate lyase. Our results show MagMOONs start blinking approximately 10 min after 2 mg/mL alginate lyase addition and this blinking is clearly detected through up to 4 mm chicken breast. This approach can potentially be employed to detect bacterial biofilm formation on medical implants by sensing specific proteases that either activate a related function or regulate biofilm formation. It can also be applied to other biosensors and drug delivery systems based on enzyme-catalyzed breakdown of gel components.

1. Introduction

Alginate gels and their derivations are widely used for implanted devices and drug delivery [1–3]. These gels can serve as local source of proteins, nucleic acids, and small molecule drugs; and the degradation of the gels will release them [4–8]. The gels are also used in wound dressings and as a platform for cell culture [9,10]. They are increasingly used in tissue regeneration by carrying and delivering proteins and cells that promote bone, muscle, cartilage and blood vessels formation [11–14].

In general, a gel allows small molecules and particles to diffuse through it, but effectively prevents the motion of particles that are large compared to the gel network mesh size. The gel can be dissolved by either breaking the crosslinks in the gel network, or enzymatically cleaving the polymer backbones allowing rapid drug release. After the gel breaks down, larger probes become free to move and the effective viscosity dramatically decreases. Detecting this rapid change in viscosity during de-gelation can also be used to determine the activity of enzymes that digest the gel. In vitro viscosity can be measured using standard viscometers such as capillary [15], plate, or falling ball viscometers [16]. To measure local viscosity in confined systems such as a cellular cytoplasm or measurement through tissue, more sophisticated methods are needed to apply force to a probe and measure its response. Möller and colleagues [17] measured the local viscoelastic moduli of the macrophages cytoplasm by recording the deflection and recovery of 1.3 μm magnetic beads when applying twisting force pulses. They also studied the intracellular phagosome transport in macrophages by monitoring the remnant magnetic field of $\sim 10^6$ phagocytized magnetic particles after initially magnetizing them with a strong magnetic field. The remnant magnetic field decayed as each particle rotated away from its initial orientation by

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independent intracellular transport forces in each cell [18]. This is an excellent non-invasive approach for intracellular investigation. However, they required 10^6 particles to measure the remnant magnetic field and the approach cannot take advantage of the single particle tracking to obtain local information of the surrounding of each individual particle. In addition to magnetometry approaches, mechanical methods based on oscillation or vibration of a cantilever have also been developed [19,20]. For example, Ehrlich and co-workers [19] designed a wireless biosensor device for early biofilm detection based on changes in the resonance frequency of a cantilever in response to change in viscosity as a polysaccharide gel was cleaved by its enzyme galactosidase. This galactosidase enzyme was designed to activate upon binding of RAP (Ribonucleic acid [RNA] III activating protein), a quorum sensing molecule generated by bacteria. When activated by RAP, the enzyme cleaved a polysaccharide substrate and produced glucose, which broke down a dextran–Concanavalin A hydrogel by competing with the dextran for binding to the Concanavalin A crosslinks. This RAP-activated gel breakdown reduced the hydrogel viscosity and was detected as an increase in the cantilever's amplitude and resonance frequency due to reduced viscous damping. This approach is sensitive but requires a power source, relatively large and complex electronics and antenna to drive the cantilever circuit and transmit the signal wirelessly.

Inspired by Ehrlich's work, we aimed to create a simple yet effective fluorescence-based sensor to detect changes in viscosity due to de-gelation activity, and monitor the fluorescence transdermally. In place of piezoelectrically driven cantilevers, we applied an oscillating magnetic field to drive the rotation of magnetic particles (MagMOONs) embedded in the gel, and measured the ability of the MagMOONs to rotate and align with the field by detecting the modulated fluorescence signal. In general, the rotational motion of magnetic particles depends upon the applied magnetic field, the magnetic moment of the particle, shape and size-dependent drag, and the viscoelastic properties of the environment. For a given set of particles the rotational motion can be used to monitor changes in the environment. If particles are optically asymmetric, the motion can be tracked optically on a single particle level (provided that the particles can be resolved, i.e. tissue does not scatter the light). For example, in 1950, Crick [21] monitored the rotational motion of micron sized aspherical particles in response to pulsed magnetic fields to measure the viscosity in chick fibroblasts. Optical tracking of rotating magnetic particles was used to monitor changes in drag during growth of single bacteria on MagMOONs [22] to monitor changes in shape of single cancer stem cells [23], to measure the viscosity of butterfly saliva [24], and to detect bacteria based on changes in viscosity when bacteria excrete biofilm polymers [25]. In addition, particle rotation has been used to track intracellular transport [26,27]. McNaughton and colleagues used asynchronous magnetic bead rotation (AMBR)-based biosensor to measure viscosity and to detect microbial growth based upon increased drag on the magnetic particles. They applied a circularly rotating magnetic field that caused the particles to rotate at the driving frequency (with a phase delay) when the rotation was slow (and/or the field is strong), or in an asynchronous rocking motion superimposed with a continuous rotating when the rotation exceeded a critical frequency: $\omega_c = mB/\kappa\eta V$, where B is the applied field, κ is a shape factor (6 for a sphere), η is the viscosity, and V is the particle volume. When the viscosity of the environment surrounding the bead changed or the effective volume changed (e.g. due to a bacterium binding onto the bead surface), the rotational period of the bead changed accordingly [25,28]. This method has high sensitivity at the single bacterium and single cell level [29]. However, the method does not work unless single particles can be resolved because each particle rotates asynchronously and no large group of particles would be aligned with each other at any given time.

Although local viscosities can be probed by optically tracking the motion of magnetically driven particles in vitro, such tracking is challenging to perform through tissue for three reasons: first, the excitation light and probe fluorescence is attenuated by the tissue; second, the tissue autofluorescence can obscure the probe signal from the probes; third, tissue scattering will cause the image to blur preventing accurate determination of the position of single particles. Fortunately, these limitations can be circumvented using MagMOONs. MagMOONs are fluorescent particles with an orientation dependent fluorescence signal generated by vapor depositing metal onto one hemisphere of a fluorescent particle and a magnetic moment that causes them to align in an external magnetic field (see Fig. 1 and Section 2). The MagMOONs feel a torque to align with an external magnetic field. If the MagMOONs are free to rotate in an oscillating external magnetic field, they blink as they flip between dim and bright orientations. Tissue does indeed attenuate the

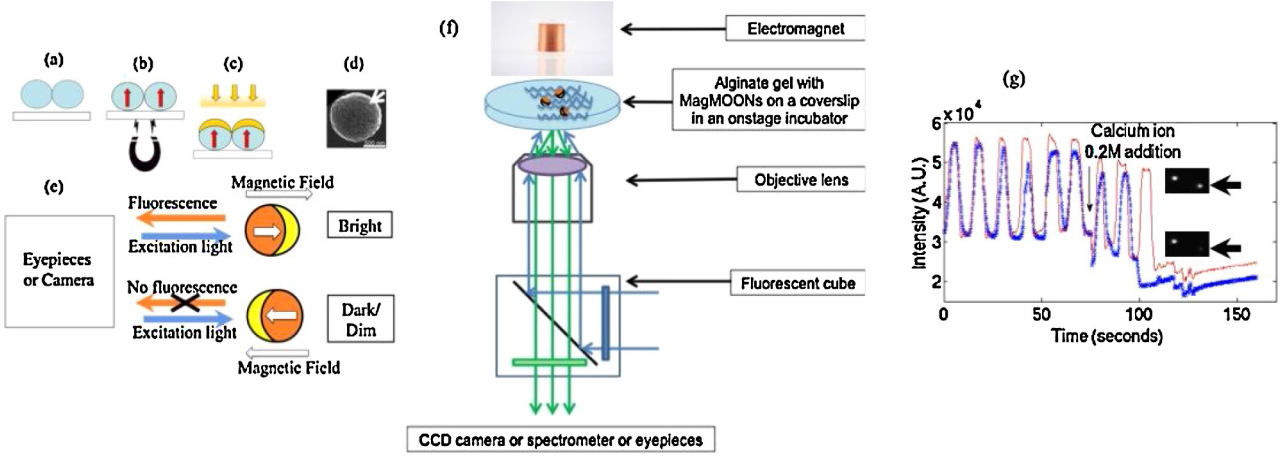

Fig. 1. Principle, setup, and example of magnetically modulated fluorescence. (a)–(d) Fabrication process: (a) 4.8 μm fluorescent ferromagnetic microspheres deposited on a glass coverslip. (b) Microspheres magnetization. (c) Metal (aluminum, gold or silver) vapor deposited onto one hemisphere of the microspheres. (d) SEM image of a Fe_3O_4 MagMOON. The arrow points to the Au-coated side. (e) MagMOON working priciple: MagMOONs blink when they rotate in response to rotating magnetic field. (f) Schematic of fluorescence micoroscopy setup. (g) Plot of the fluorescence intensity of two single MagMOONs blinking before and after adding 400 μL $CaCl_2$ 0.2 M to 400 μL alginate 10 mg/mL (at around 75 s). MagMOONs stop blinking about 30 s after $CaCl_2$ addition, although one MagMOON (blue star) stops blinking one cycle before the other (red line). Inset shows one frame where both MagMOONs are bright, and one frame where one MagMOON is trapped in the dim orientation, while the other is still able to rotate back to the bright orientation during its last cycle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)