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Magnetic microdrill as a modulated fluorescent pH sensor

Brandon Howard McNaughton, Jeffrey N. Anker, Raoul Kopelman*

University of Michigan, Department of Chemistry, 930 North University Ave., Ann Arbor, MI 48109-1055, USA

Abstract

Remotely exploring biological tissues using magnetically driven micromachines could one day allow for medical diagnostics and treatment. We developed a magnetically driven drill capable of sensing pH changes. The drill is coated on one side with a fluorescent indicator dye that blinks when the drill rotates; extracting the blinking signal reduces background interference by over a factor of 100.

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Keywords: Sensors; Microdrill; MagMOONs; Spectroscopy; Nanotechnology; pH sensor; Fluorescence; Blinking signal

1. Introduction

Many diseases have chemical signatures that can be used for diagnosis, study and treatment. For example, measuring pH and pO_2 (oxygen pressure) concentrations could reveal important information about cancerous tumors and their response to treatment [1]. One way to measure chemical concentrations and gradients is to use endoscopically guided chemical sensors, which allows in situ measurements [2]; however, endoscopic probes are often large, invasive, and difficult to manipulate. Adding magnetic control to these endoscopes allows them to be guided more easily [3].

Magnetic control allows remote guidance of untethered vehicles for local drug delivery and hyperthermia applications [4]. Magnetic microdrills and magnetic balls can be moved in three-dimensional patterns without wire or fiber attachments [3–5]. Magnetically driven microdrills were developed by Honda et al. in 1996 [5], and subsequently shown to drill through bovine tissue [6], swim through intestine phantoms [7], act as a local heat source for hyperthermia [4], and enable controlling multiple drills independently, based on differing maximum rotation rates [8]. Presented here is a novel technique that combines advantages of remote magnetic guidance with in situ chemical sensing,

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*Corresponding author. Tel.: +1 734 764 7541; fax: +1 734 936 2778.

E-mail address: kopelman@umich.edu (R. Kopelman).

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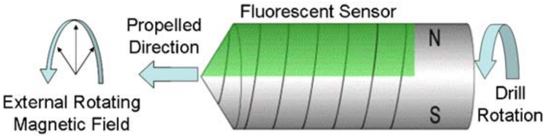

Fig. 1. Schematic of a modulated fluorescent magnetic microdrill, where the green shows the fluorescent sensor.

showing promise for study and monitoring of diseased tissues.

The chemical sensor on the microdrill is designed to make accurate measurements in protein solutions by encapsulating fluorescent dyes in a semipermeable matrix that insulates the dye from the environment but allows small analytes to pass through. The matrix also allows multiple dyes and materials to be added to the same sensing medium, allowing synergistic sensing schemes for the detection of more types of analytes, based on ion correlation or enzyme reaction. To date, our group has fabricated Ca^{2+}, Zn^{2+}, Cu^{+}, Mg^{2+}, K^{+}, Na^{+}, Cl^{-} and other ion probes. Additionally, we have fabricated optical nanoprobes for dissolved gases such as oxygen and NO, small molecules such as glucose, and radicals such as OH [9–12]. These probes could be easily adapted to the drill. In addition, the drill is made to blink as it rotates so that its signal stands out from the background, similar to magnetically modulated optical nanoprobes, MagMOONs, also developed in our lab [13,14].

Using magnetic modulated microdrills as a dynamic sensing platform allows simultaneous drilling and physicochemical probing and characterization of the surrounding environment. A conceptualization of this is shown in Fig. 1. As proof of principle, a 3.2 mm diameter pH sensing drill was fabricated, used to probe a gelatin environment, and used to monitor pH through a discontinuous pH change. We were also able to modulate the drill's fluorescent signal, which allowed for a background reduction on the order of 100.

2. Fluorescent magnetic microdrill (method)

The basic components of a magnetic microdrill consist of a spiral-type blade for propulsion and a magnetized body to deliver a torque to rotate and propel it. For example, Fig. 1 shows a cylindrical magnet attached to a spiral blade, and magnetized through its diameter. When an external field is applied, the magnet will orient so that it aligns with the external magnetic field. This minimizes the magnetic torque provided by the external field, which is given by $\tau = MB\sin(\theta)$, where τ is the magnitude of the torque acting on the drill, B is the strength of the external magnetic field, M is the magnetization of the drill, and θ is the angle between M and B. When the external magnetic field rotates, the drill rotates and the screw propels it forwards or backwards, depending on the direction of rotation. Magnetic torque causes the drill to orient perpendicular to the external magnetic rotational plane. The direction of translation is perpendicular to the plane that the external magnetic field makes as it rotates. The torque is delivered to the magnetic drill through this external magnetic field, allowing the mechanism to swim (or drill) with no attachments. This, for example, would allow the drill to enter and exit a system at different locations.

A prototype magnetic microdrill is shown in Fig. 2a. We used a rectangular NdFeB magnet (Dexter Magnets Technology, Chicago, IL) for our magnetic body (~2.0 mm × 2.0 mm × 4.5 mm) and spiral brass wood screw that has a diameter of 3.2 mm as the spiral blade. The head of the screw was sawed off and the thread was attached to the rectangular magnet. To attach the thread to the magnet, heat shrink tubing was placed over the two and heated. The rectangular magnet will incur

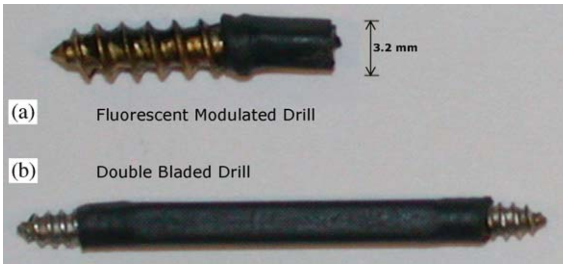

Fig. 2. Photographs of magnet microdrills. Note that the heat shrink holds the blade to the magnet. (a) A single bladed fluorescent modulated microdrill. (b) A double bladed microdrill without fluorescent sensing element.

more drag than a cylindrical magnet. To reduce drag, we extended the heat shrink so that it encompassed the entire magnet, which made it more cylindrical.

We also fabricated a “double-headed” drill shown in Fig. 2b. This drill is double-bladed and would allow a microdrill to drill its way out of an environment through the use of only an external magnetic field. The fabrication for this microdrill was similar to the previous mentioned drill, but instead utilized two #2-size screws (Small Parts Inc., Miami Lakes, FL).

To this basic drill platform, various components and functions can be added. To allow the drill to sense, a fluorescent pH sensitive dye in a plasticized polymer matrix was coated onto the surface of the spiral blade. Two different pH sensitive dyes in two matrices were used as coatings, namely carboxynaphthofluorescein (CNF) in poly (methyl methacrylate) [12,15] and ETH 5350 in a polyvinylchloride (PVC) matrix [16]. Each solution included a plasticizer, namely nitrophenyl octyl ether for the methacrylate and a 2 to 1 ratio of dioctyl sebacate (DOS) to PVC. The spiral part of the drill was dip coated in one of the above solutions. Fig. 3 shows the coated drill with the methacrylate matrix. After the solution dried, we blackened half of the spiral blade with a permanent marker. This allowed for modulation when the drill was rotated. Once the modulated fluorescent microdrill was constructed, it was placed into a gelatin environment, where we could perform background subtraction and pH monitoring.

The pH is determined by measuring the ratio between acid and base peaks of the sensor coating on the drill. The drill was previously characterized by placing it in a series of pH buffers (Fig. 5). Both the CNF and ETH5350 pH sensitive dyes are ratiometric, meaning that the spectrum of the fluorescence has two peaks and the ratio between these peaks changes as the pH levels change. Magnetic fields will both drive the drill forward (or backwards) and rotate the drill, causing the observed fluorescence to change from “off” (opaque ink side is visible) to “on” (fluorescent side is visible). This modulation allows sensor fluorescence to be separated from fluorescent backgrounds.

For measurements of changing pH levels, we prepared two different gelatins: one of pH 2.0 and one of pH 11.5. The gelatin samples, purchased from Sigma, were dissolved in 100 mM phosphate buffer solutions at 100 °C at varying concentrations from 0.5–2% weight/volume, placed in 50 ml centrifuge tubes, and cooled down overnight in a refrigerator at 6 °C. The gelatin from one tube was then placed in contact with gelatin in another tube so that the drill could pass from one environment to another, creating a physical and pH discontinuity.

The drills were observed through an Olympus IMT2 epifluorescence microscope and imaged with a Roper CoolSnap ES CCD camera, and a Nikon Coolpix 995 digital camera. Spectra were acquired with an Acton Research Corp spectrometer with a Hamamatsu 230 CCD, all interfaced to a Pentium computer. A mercury lamp and a xenon lamp were used as excitation sources, with an Olympus blue filter cube. A program written in LabView (National Instruments, Austin, TX) controlled the external rotating magnetic field and interfaced our spectrometer to the computer. The external magnetic field was generated by using a cylindrical

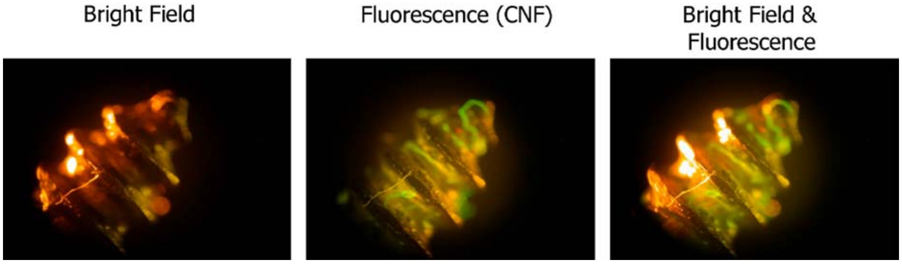

Fig. 3. Photographs of the magnetic microdrill coated with CNF and poly (methyl methacrylate).

NdFeB magnet with a diameter of ~1.6 cm, magnetized along its diameter (Dexter Magnets Technology, Chicago, IL), and attached to a stepper motor. When the stepper motor is activated the cylindrical magnet rotates, and thus produces a rotating magnetic field to drive the microdrill.

3. Results and discussion

3.1. Modulation and background subtraction

For our modulation and background subtraction experiments, we used the polymethacrylate and CNF coating solution. The drill was placed in a gelatin environment and the mercury lamp, used to excite the fluorescent dye, created a background. A program was used to alternatively orient the microdrill "on" and "off", acquire a series of spectra for each orientation, and subtract "off" from "on". Subtraction was performed in order to eliminate the background and is shown in Fig. 4. A mercury lamp peak at 800 nm, from reflection in the cube and partly reflection from the sample, caused background interference. The initial background intensity at 800 nm was approximately 2 arbitrary fluorescence units (Fig. 4a). After background subtraction, the 800 nm background was reduced to below 0.02 (Fig. 4b), resulting in a 100-fold reduction in background. This value is not an inherent limitation [13]. Using the same procedure will allow sensing in biological systems in the presence of strong autofluorescent backgrounds. Additionally, when light passes through biological tissue there will be scattering of the emitted fluorescence. Despite the presence of scattering, a modulated signal could still allow for background subtraction.

The polymethacrylate and CNF coated sensing microdrill provided a demonstration of simultaneous drilling and background subtraction, although the dye matrix combination was not very reliable or sensitive to changes in pH. For subsequent experiments, a more sensitive coating, ETH 5350 dye and PVC matrix solution, was applied to the drill. With this more hydrophobic coating, we have successfully monitored changes of pH as discussed below.

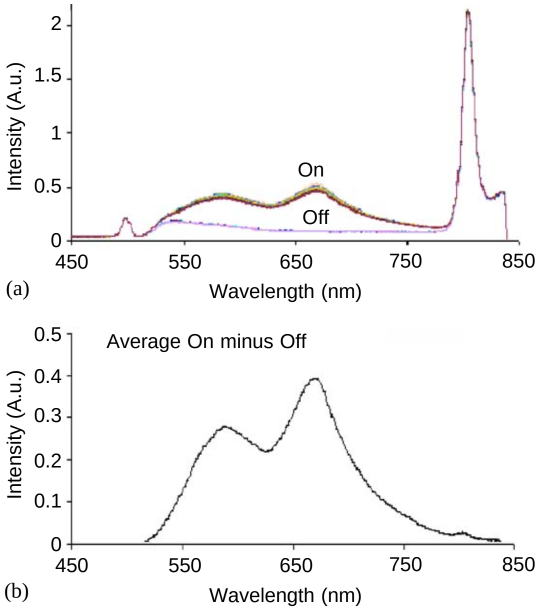

Fig. 4. Spectra from pH sensing microdrill coated with CNF and poly (methyl methacrylate). (a) The graph shows a sequence of spectra for the drill in "on" and "off" orientation. (b) The graph is obtained by subtracting average "off" from average "on" and thus reducing background evident at 800 nm.

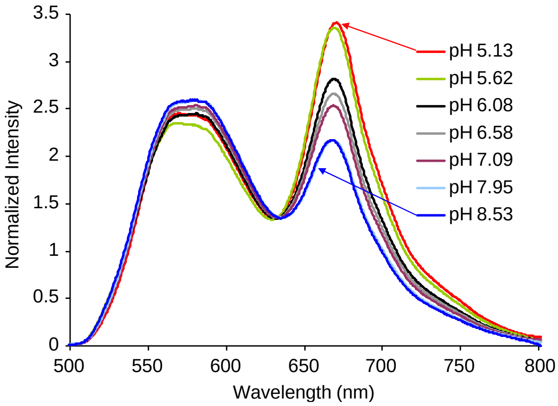

Fig. 5. The spectral response to pH of the ETH 5350 and PVC coating from a series of buffers from a pH of 5.13 to a pH of 8.53. A common background was subtracted from each spectrum.

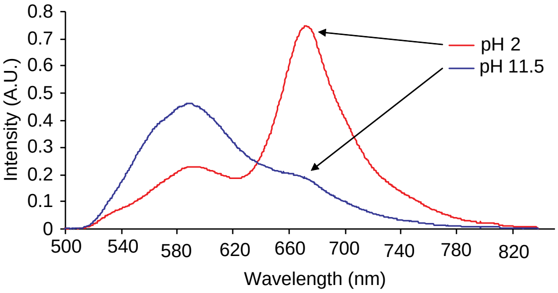

Fig. 6. The spectra of the ETH 5350 and PVC coating changed as we drilled from a gelatin at pH 2 to a gelatin at pH 11.5.

3.2. Sensing a discontinuous pH change

The ETH 5350 and PVC coated microdrill was sensitive to changes in pH, as shown in Fig. 5. The spectral response to pH was obtained by placing the drill in a series of pH buffers and subtracting a common background. To observe the pH change while drilling, two cylindrical blocks of gelatin, prepared at with pH 2 and pH 11.5 buffers respectively, were placed in contact as described in section 2. The interface between the two gelatin blocks created both a pH discontinuity and a physical discontinuity. We drilled through this discontinuity and examined the fluorescent spectra in each gelatin. It is clear from Fig. 6 that the peak ratios changed drastically as we drilled from a gelatin of pH 2.0 to a gelatin of pH 11.5.

The large change from pH 2.0 to 11.5 is outside the range of most biological environment, but the same principle of measuring a pH change while drilling also applies to measuring the pH change within the range of biological tissue as shown in Fig. 5. Enabling a magnetic microdrill to measure pH levels using a fluorescent indicator creates new possibilities. In addition to pH sensing, the drill could be coated with various indicators to allow for measurements of oxygen, calcium, and other elements and molecules [9–12].

Additionally, the double bladed drill (Fig. 2b), was able to drill into, through, and out of a gelatin environment controlling the drill only with external magnetic fields. This allows for entering and exiting a system at two unique locations. Also, the spatial separation of the two spiral blades could allow for multiplexing where one blade could be coated with a pH sensitive indicator and the other blade coated with an oxygen sensitive indicator.

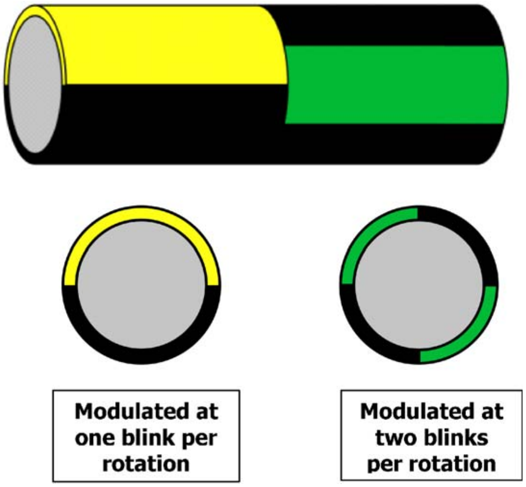

Fig. 7. Schematic of a drill capable of sensing multiple analytes, where the signal from each analyte blinks once, twice, or multiple times per revolution.

4. Conclusion

We have shown that a magnetic microdrill can be fabricated so that it can characterize its environment as it drills and, upon modulation, the background fluorescence can be subtracted and reduced to below 1% of its original level. This allows measurements of chemical concentrations as a function of position. Remotely monitoring chemical concentration gradients could be used for in vivo measurements of biological tissues. Further development of fluorescent magnetic micromachines will lead to miniaturizations that allow less invasive sensing or probing of smaller volumes. We expect that drills could eventually be miniaturized down to a size similar to biological propellers, such as 20 nm bacterial flagella [17] based on torque, drag, and energy calculations. In addition, drills could be prepared with different chemical indicators that blink at different frequencies, which thus can be used for simultaneous sensing of multiple types of chemicals, as shown schematically in Fig. 7. Preliminary experiments suggest that magnetically modulated fluorescent (or reflective) microdrills can also be used as roving light sources and as in situ viscometers.

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