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Self-Powered Sensor for Naked-Eye Detection of Serum Trypsin

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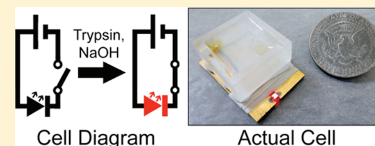
S *Supporting Information*

ABSTRACT: Here, we report a device for the detection of the proteolytic enzyme trypsin, which is a biomarker for pancreatitis. The sensor is self-powered, easy to use, and signals the presence of trypsin via a light-emitting diode (LED) that is visible to the unaided eye. Assay time is ~3 h, and the limit of detection is 0.5 μg/mL, which is within the range required for detection of trypsin at levels signaling acute pancreatitis. The sensing mechanism relies on trypsin digestion of a gelled protein layer. Partial digestion of the protein layer permits hydroxide penetration and subsequent etching of an underlying Al membrane. Degradation of both the protein and Al layers exposes an underlying Mg anode and closes an electrochemical circuit that produces ~2.2 V. This is sufficient voltage to illuminate the LED. A logarithmic relationship is observed between the time required for LED illumination and trypsin concentration. The device is equally effective for trypsin dissolved in buffer or serum media.



Here, we report a self-powered device for detection of trypsin in serum at pathological concentrations that uses a light-emitting diode (LED) to deliver a response visible to the unaided eye. The assay is complete within ~3 h, and it provides quantitative results without requiring an external power source. The device, illustrated in Scheme 1, is an Mg//Fe^{3+} galvanic cell with protein and Al passivating layers blocking Mg oxidation at the anode. In the presence of trypsin and hydroxide, however, these passivating layers are etched, and this action connects the anode and cathode, thereby illuminating the LED. However, in the absence of trypsin, the protein passivating layer blocks LED illumination. In this article, we show dose−response assays using trypsin in both buffer and serum, and we report a relationship between the concentration of trypsin and the LED illumination time. The limit of detection is 0.5 μg/mL, even in the presence of the blood-borne proteins, sugars, and salts found in serum. The novelty of this single-use sensor arises from three factors. First, it is self-powered by the Mg//Fe^{3+} galvanic cell discussed earlier. Second, the presence of trypsin is reported by an LED, and therefore, only the unaided eye is required for detection. Third, the device is simple to operate.

Trypsin is a digestive enzyme produced in the pancreas that cleaves proteins on the c-terminal side of arginine and lysine residues. It is most active under slightly alkaline conditions and in the presence of Ca^{2+}, Mg^{2+}, and Mn^{2+}.[1] Trypsin is formed initially as the proenzyme trypsinogen, which self-cleaves to yield the more active form as needed.[2] This self-regulating process can be adversely affected by pathologies, such as pancreatitis, which result in organ damage and release of enzyme into the blood. An immunoassay-based, quantitative study found healthy individuals to have a mean serum trypsin concentration of 0.25 ± 0.1 μg/mL, whereas acute pancreatitis patients exhibited a higher concentration of 1.4 ± 0.6 μg/mL.[3] Speed is a factor to consider when trypsin was used as an indicator of pancreatitis. For example, a time-resolved enzyme linked immunosorbent assay (ELISA) study found that the serum trypsin concentration is at a maximum 24 h after the onset of symptoms, but it is decreased at 48 h and again at 72 h.[4] For diagnostic detection, a system must be sensitive to ~1 μg/mL enzyme, capable of distinguishing between small variations in concentration, and sufficiently accessible so as to be implemented immediately after the appearance of pancreatic symptoms.

Ionescu et al. have published an indirect electrochemical method for trypsin detection using a catalyst-modified Pt disk electrode that generates an electroactive species that can be detected by cyclic voltammetry.[5] In this study, electrodes were modified with electropolymerized glucose oxidase (GOx) and coated with a layer of gelatin. When immersed in a solution containing trypsin (9380 U/mg) and glucose, the enzyme digested the gelatin layer, and GOx converted glucose to electroactive peroxide.

The sensing device described here relies on an LED to signal the onset of an electrochemical reaction. Previous electrochemical studies have used LEDs to report the passage of current in a battery[6] and as a component in more complex circuits to interrogate and quantify electroactive species under external potential control.[7,8] The LED used here has a minimum turn-on voltage of ~1.7 V and is self-powered by the two half cells comprising the sensor. Specifically, Mg oxidation and Fe^{3+} reduction yield an observed cell voltage of ~2.2 V. Self-powered sensors have been reported previously. For example, biofuel cells developed by Willner,[9] Minteer,[10] and their co-workers use anodes modified with enzymes or mitochondria to detect sugars and nitroaromatics, respectively.

In this article, we present a method for the indirect detection of trypsin in serum. The limit of detection is ~0.5 μg/mL, which is sufficiently low for detection of pathological conditions.

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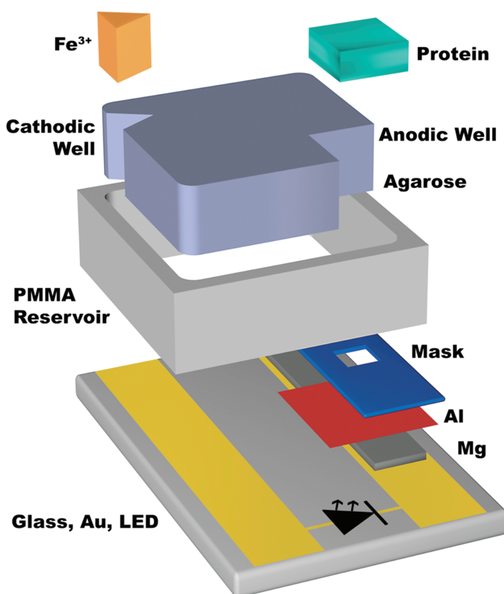
Scheme 1



The device provides a logarithmic response that spans more than 2 orders of magnitude in trypsin concentration, regardless of whether the analyte is present in buffer or serum. Detection is achieved with just the unaided eye within 3 h of analyte injection. Finally, the device is fully self-powered and, therefore, appropriate for many point-of-care applications, including those in remote locations.

EXPERIMENTAL SECTION

Chemicals and Materials. Trypsin from porcine pancreas (Type IX), gelatin, Type A, 300 bloom electrophoresis grade from porcine skin, heat-inactivated and sterile filtered equine serum, and calcium acetate monohydrate (>99%), henceforth referred to as CaAc, were purchased from Sigma (St. Louis, MO). Genetic-analysis grade agarose and N_{α}-*p*-tosyl-L-arginine methyl ester (TAME) hydrochloride were obtained from Thermo Fisher Scientific (Waltham, MA). BiPRO whey protein isolate, henceforth referred to as WPI, was purchased from Davisco Foods International (LaSueur, MN). Magnesium alloy AZ31B was obtained in 1 mm-thick, flat sheets from A1 Alloys (San Diego, CA). This alloy is ~95% Mg and has electrochemical properties similar to those of pure Mg. Additional materials used in this study are described in the Supporting Information.

Cell Assembly. Components of the sensing platform are shown in Scheme 1. The device components are labeled and displaced vertically with respect to order of assembly. Specific details on component assembly are provided in the Supporting Information.

Gelatin and WPI were selected as the components of the protein layer. Protein solutions were prepared in 0.7 M HEPES buffer at pH 7.55. The concentrations of the proteins were individually optimized for buffer and serum. For buffer-based assays, the protein solution contained 5% gelatin and 10% WPI (both w/v). For detection of trypsin in serum, the protein solution contained 6% gelatin and 9% WPI (both w/v). The protein powders were added to solution in conical tubes and dispersed using a vortex mixer. The tubes were placed in a sonicating water bath at 60 °C for 15 min to dissolve the proteins and defoam the solution. During the preparation and handling of the protein solutions, it was important to limit bubble formation to avoid pinholes that could lead to premature etching of the Al foil. After sonication, the molten protein was aliquoted and stored at 4 °C until needed. Just prior to running an assay, these aliquots were placed in a sonicating bath at 60 °C for 2 min, and then, 250 μL of the solution was added to the anodic well of each device. Cathodic wells were filled with 50 μL of 0.2 M $FeCl_3$. The devices were then chilled at 4 °C for 30 min to gel the protein. Devices were inspected before use, and those having a smooth, bubble-free protein layer and a nonilluminated LED were retained for assays.

Trypsin Assays. The assays were carried out using 100.0 μL volumes of buffer or serum with or without added trypsin. The evaluation of the activity of trypsin is described in the Supporting Information. Stock trypsin solutions were prepared daily at a concentration of 10.0 mg/mL in either buffer or serum and diluted to concentrations ranging from 0.50 to 100 μg/mL. Buffer trials used trypsin in 46 mM, pH 8.0 TRIS containing 11.5 mM CaAc. For serum trials, trypsin was diluted into 100.0 μL of equine serum having a native pH of 7.9, and then, an additional 13.0 μL of 0.115 M CaAc was added. Negative control assays used 100.0 μL volumes of buffer or serum, with an additional 13.0 μL of 0.115 M CaAc added to the serum assays. The test solution was added to the anodic well of each device, which was then placed in a closed Petri dish inside an air incubator at 26 ± 1 °C and left for 2 h. During incubation, the incubator maintained a constant temperature compatible with the stability of the protein layer and the activity of trypsin while ensuring uniform conditions for all assays. After incubation, an additional 50.0 μL of 0.20 M Fe^{3+} solution was added to the cathodic well to compensate for solution sorbed into the gel during incubation. Devices having an illuminated LED at this stage were discarded. Finally, the Al dissolution process was initiated by adding 300 μL of 1.0 M NaOH to the anodic well. The time between hydroxide addition and LED illumination was recorded.

RESULTS AND DISCUSSION

Device Overview. This trypsin sensor is designed around an electrochemical cell composed of two half cells in which Fe^{3+} is reduced and Mg is oxidized. Prior to adding the analyte, the two half cells are prevented from discharging by the presence of Al and protein barrier layers positioned above the Mg anode (Scheme 1). However, when trypsin is added to the anodic well, it degrades the protein layer. Subsequent addition of hydroxide etches the Al passivating layer,[11] and this exposes the Mg anode to the KCl-impregnated agarose salt bridge.[12] Now, the two half cells are in electrochemical contact, and sufficient voltage is produced to illuminate an LED. Hence, the LED signals the presence of trypsin. If the added analyte does not contain trypsin, then the protein layer remains intact and retards transport of hydroxide to the Al layer. Therefore, the electrochemical circuit remains open, and LED illumination is prevented. More detailed information about device operation is provided later.

Dose−Response Assays. Dose−response assays were carried out for trypsin in buffer and serum. The assay was initially optimized using trypsin in buffer, and then, the protein gel was reoptimized for detection of trypsin in serum. Specifically, the amount of gelatin was increased from 5% to 6%, and WPI was concomitantly decreased to keep the total amount of protein constant. The additional gelatin used for the serum assays prevented delamination of the protein layer from the Al, which was observed only when serum media was used.

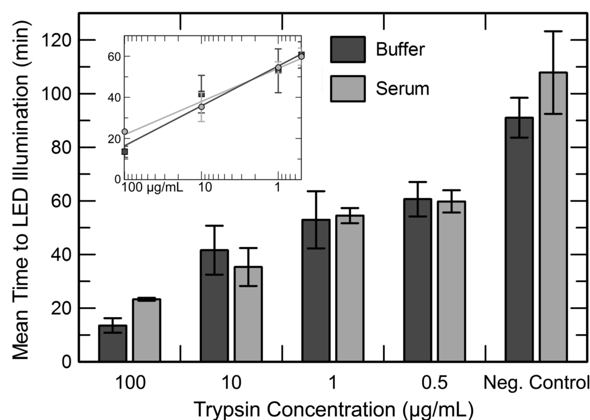
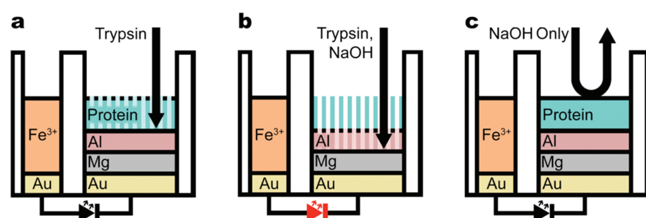


Figure 1. Histogram correlating the average time between addition of hydroxide and illumination of the LED to the concentration of trypsin present in buffer or serum. The negative control was obtained for a trypsin-free sample. The inset is a semilog, least-squares plot correlating the average illumination time to the concentration of trypsin. Error bars represent one standard deviation from the average of data obtained for 3−5 independently prepared sensors.

Scheme 2



Data from the trypsin assay in buffer and serum are shown in Figure 1. Here, the average time between addition of hydroxide and LED illumination time for 3−5 different devices is plotted against the different trypsin concentrations and a trypsin-free negative control. The error bars represent one standard deviation from the mean. It is important to note that even for the lowest trypsin concentration (0.50 μg/mL) the error bars do not overlap with the negative control assay. The inset in Figure 1 shows that there is a linear relationship between the mean time for LED illumination and the logarithm of the trypsin concentration. The R^2 values for the buffer and serum fits are 0.96 and 0.99, respectively. Because the inset plot is concerned with the response of the system to changes in trypsin concentration, the trypsin-free, negative control data are not included.

Role of the Protein Gel and Al Barrier Layers. A diagram illustrating the roles of the protein and Al barrier layers is shown in Scheme 2. In panel a, the protein gel layer is exposed to a solution containing trypsin. Trypsin partially digests the protein layer, which renders it more permeable to hydroxide than the native gel. After trypsin digestion for 2 h, hydroxide is introduced into the anodic well. Panel b shows that hydroxide penetrates the digested protein layer and partially dissolves the Al layer to expose the Mg strip. This closes the electrical circuit and results in LED illumination. However, in the absence of trypsin digestion (panel c), migration of hydroxide through the gel layer is slowed and, therefore, LED illumination is delayed.

The difference in illumination times arises from the properties of the two proteins used in the barrier layer. Gelatin gels by heat-induced (>50 °C)[13] denaturation followed by hydrogen-bond-driven coalescence.[14] However, gelatin is unstable in the presence of hydroxide, so WPI is added to improve its tolerance to basic conditions. WPI stabilizes the gel under basic conditions, because it denatures at alkaline pH, thereby exposing buried cysteine residues that can polymerize via disulfide bonding[15−17] to form a rigid, proteinaceous solid.[18,19] Once polymerized, a WPI-containing gel is hydroxide resistant unless it has previously been treated with trypsin to degrade the gelatin component.[20,21] In this latter case, hydroxide moves through the protein layer and then encounters and degrades the Al barrier. In the absence of trypsin, the added base polymerizes WPI and rigidifies the gel, thereby increasing the time required for base attack on the protective Al membrane. The key point is that the rate of hydroxide transport through the gel depends on the concentration of trypsin (Figure 1).

SUMMARY AND CONCLUSIONS

We have described a self-powered trypsin sensor that is easy to use, detects trypsin at pathological concentrations in serum, and signals detection with an LED in ~3 h. The range of trypsin concentrations that can be quantitated includes those that are characteristic of acute pancreatitis. The discharge of the galvanic cell is gated by the two-component protein gel. That is, in the presence of trypsin, the gel is degraded and this results in faster transport of hydroxide and subsequent corrosion of the underlying Al barrier. In the absence of trypsin, however, base polymerizes the WPI component of the protein layer and increases the time required for hydroxide to etch the Al barrier. Another important aspect of this sensor is that it is self-powered. In essence, the device is a battery having a trypsin-selective switch that closes the circuit between the anode and cathode. The sensor works equally well in buffer or serum, demonstrating the resiliency of this method to the endogenous proteins, sugars, and salts present in real biological fluids.

ASSOCIATED CONTENT

Supporting Information. Additional information about the chemicals and materials, instrumentation, cell assembly, enzyme activity assay, and controls. This material is available free of charge via the Internet at http://pubs.acs.org.

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