An implantable wireless biosensor for the immediate detection of upper GI bleeding: a new fluorescein-based tool for diagnosis and surveillance (with video)

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Background: Early recurrent hemorrhage after endoscopic intervention for acute upper GI bleeding (UGIB) can approach 20% and leads to increased morbidity and mortality. Little has changed over the past several decades regarding immediate posthemorrhage surveillance, and there has likewise been no significant improvement in outcomes.

Objective: To develop and test an endoscopically implantable wireless biosensor for real-time detection of fluorescein-labeled blood in ex vivo and in vivo porcine models of UGIB.

Setting: Animal laboratory.

Design: Benchtop and acute animal studies.

Subjects: Five pigs.

Interventions: UGIB models were surgically created in living pigs. Biosensors were endoscopically deployed in the stomach using standard endoscopic clips. The ability to detect acute UGIB and estimated blood loss leading to biosensor activation were recorded. Feasibility of wireless data transmission out of the body to an external computer and cell phone was assessed.

Main Outcome Measurements: Technical feasibility and immediate complications.

Results: A porcine UGIB model was successfully created. Biosensors were able to detect all acute bleeding events and wirelessly transmit out of the body, and successfully sent an emergency text message to the intended cell phone in all cases. Average estimated blood loss leading to biosensor activation was 30 mL (10–75 mL).

Limitations: Animal study; small numbers.

Conclusions: An endoscopically implantable wireless biosensor successfully detected acute hemorrhage in a porcine UGIB model and sent an emergency cell-phone alert in real time.

Rebleeding remains problematic for the 300,000 patients who are hospitalized yearly in the United States with upper GI bleeding (UGIB), for which all-cause mortality ranges from 5% to 19%.[1-4] The most common etiologies include peptic ulcer disease, esophageal varices, and erosive conditions (gastritis, esophagitis, duodenitis).[5-7] After endoscopic therapy of nonvariceal upper GI hemorrhage, the rate of in-hospital rebleeding has been reported to be

Abbreviation: UGIB, upper GI bleeding.

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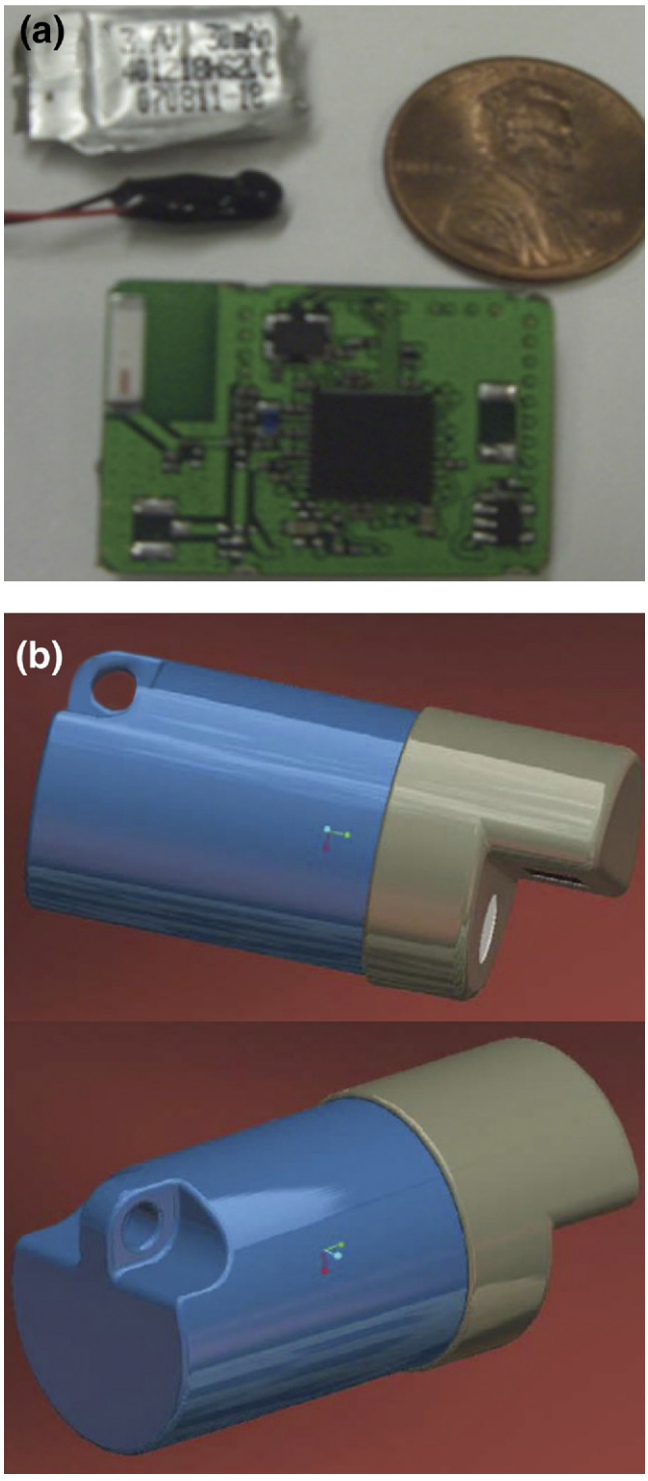

Figure 1. Biosensor device. **A**, Internal components. **B,** External casing.

as high as 32% in a single-center study.[8] However, rebleeding rates are typically thought to be in the 10% to 16% range.[4,7,9-11] After endoscopic variceal ligation in acute variceal hemorrhage, rebleeding within 5 days occurs in as many as 15% of cases.[12] Several studies have shown that both variceal and nonvariceal rebleeding, and

Take-home Message

- An endoscopically implantable wireless biosensor for immediate detection of upper GI bleeding succeeded in a porcine bleeding model.

in particular in-hospital rebleeding, to be strong predictors of in-hospital mortality and overall mortality.[13-15]

Current clinical options for the detection of rebleeding include vital signs monitoring, serial hematocrit/hemoglobin checks, and observation of clinical status (eg, melena, hematemesis).[16] However, these methods can be imprecise and often require clinical interpretation.[17,18] Furthermore, these methods usually do not indicate a re-bleeding event in real time and sometimes call attention to a rebleeding event after significant blood loss has occurred.[19]

The aim of this proof-of-principle study was to develop an endoscopically implantable wireless biosensor for real-time detection of UGIB and evaluate operating characteristics and technical feasibility in in vivo porcine models.

METHODS

Wireless biosensor

The size of the wireless biosensor used in this study was 15 × 9 mm (Fig. 1). It was equipped with a proprietary

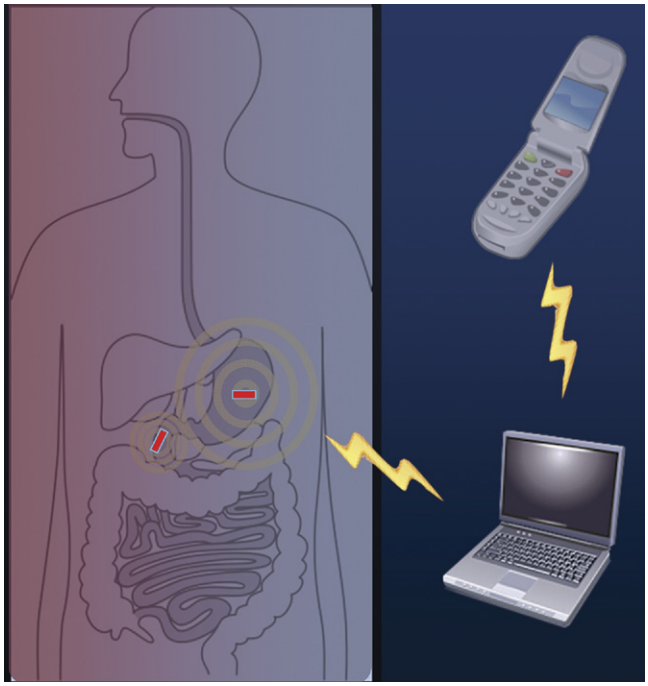

Figure 2. Wireless communication schema: internal biosensors link wirelessly with each other as well as external computers or transceivers that can, in turn, relay data to the patient's cell phone or an emergency response network.

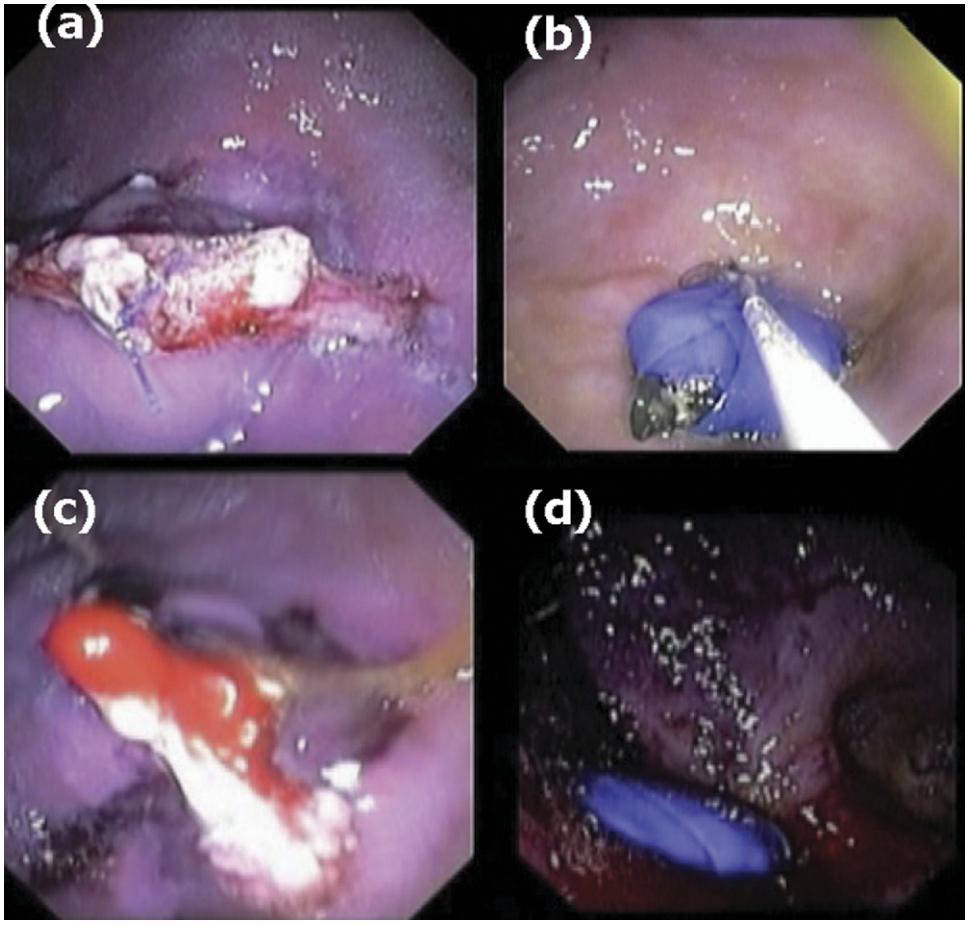

Figure 3. Endoscopic images in acute porcine study. **A**, Exposed gastroepiploic artery. **B**, Endoscopic deployment of biosensor device. **C** Lacerated artery with resultant bleeding. **D**, Biosensor bathed in blood.

sensor component, a central processing unit characterized by ultralow power consumption, a radio and antenna capable of transmitting out of the body, and a battery able to operate for days or up to a week. The biosensor was designed to detect blood labeled with fluorescein, a U.S. Food and Drug Administration–approved, intravenously injected fluorophore that remains systemically available for as long as 48 to 72 hours.[20] This design feature was critical in allowing the biosensor to differentiate between old blood and new blood. On biosensor activation, a wireless signal was transmitted out of the body to an external transceiver. The operation of the system was controlled by a computer that modifies sensing parameters, monitors serial readings, generates a plot of the bleeding signal versus time, and wirelessly relays the information of a bleeding event to a cell phone. Several biosensors could be used at once, in essence creating a wireless medical network (Fig. 2). For details regarding benchtop prototype development, see Fig. 5 and Appendix (available online at www.giejournal.org).

Acute animal study

Animal labs were conducted at Concord Biomedical Sciences and Emerging Technologies (Lexington, Mass) after approval from the Institutional Animal Care and Use Committee.

In vivo porcine studies were performed in 5 Yorkshire pigs weighing approximately 40 kg. After induction of anesthesia using intramuscular injection of Telazol (tiletamine/zolazepam) 4.4 mg/kg, xylazine 1.1 mg/kg, and atropine 0.05 mg/kg (all medications from Fort Dodge Animal Health, Fort Dodge, Iowa), the pigs were intubated and maintained under isoflurane 1% to 4%. An UGIB model was surgically created in each pig using a modification of a previously published technique.[21] A laparotomy was performed to dissect the gastroepiploic artery and transmurally fix it across the anterior gastric wall. The resulting endoscopic appearance was similar to a large Dieulafoy lesion (Fig. 3A).

Subsequently, a single biosensor was endoscopically implanted in the antrum using a double-channel therapeutic gastroscope (GIF-2T100; Olympus Medical Systems, Center Valley, Pa). A suture loop was first attached to the biosensor, and the device was back-loaded on the endoscope tip with the suture being held in the jaws of a Resolution clip (Boston Scientific Corp, Natick, Mass). The sensor was then clipped to the antrum via the suture loop (Fig. 3B).

Figure 4. External computer displaying signal versus time plot.

One to 4 hours after fluorescein injection (fluorescein 500 mg intravenously, normal adult human dose,[20] diluted in 100 mL of normal saline solution), the exposed gastroepiploic vessel was lacerated using rat-tooth forceps (Fig. 3C). Biosensor data were transmitted to a transceiver connected to a laptop computer positioned 5 feet away. This computer, in turn, sent a wireless text message to a cell phone denoting a bleeding event. The volume of blood leading to biosensor activation was estimated by aspirating the gastric contents (Fig. 3D) and performing a back-calculation using total volume and hematocrit (blood volume = total volume of aspirate × hematocrit). Animals were subsequently euthanized.

RESULTS

Porcine UGIB model was successfully created in all 5 animals (Video 1, available online at www.giejournal.org). The biosensors were successfully affixed to the antrum in all 5 animals. Biosensors were able to continuously transmit data out of the body to an external laptop computer (Fig. 4), which then relayed an emergency text message to the intended cell phone in 5 of 5 animals (100%). The mean estimated blood loss for detection was 30 mL (range 10-50 mL).

DISCUSSION

Many studies have shown that the majority of patients who rebleed within 30 days after endoscopic hemostasis and high-dose proton pump inhibitor therapy do so within the first 72 hours.[22-24] Unfortunately, posthemorrhage surveillance methods have not changed in decades. Although most of these patients are still hospitalized at the time of recurrent hemorrhage, detection is often a slow and imprecise process. For example, melena could represent passage of either old or new blood. Serial hematocrit checks can be confounded by dilution from aggressive volume resuscitation. Because early intensive resuscitation has been shown to decrease mortality after acute UGIB,[25] an early-warning remote surveillance system for rebleeding may be clinically beneficial. We have developed an endoscopically implantable wireless biosensor that can potentially provide an early-warning surveillance system for hospitalized patients within this important timeframe.

The biosensor is designed to detect fluorescein, which carries certain advantages as a proxy for rebleeding. First, a fluorescein-based system allows sensitive detection of an exogenous substance and, therefore, differentiation between old blood and fresh blood, which is critical in

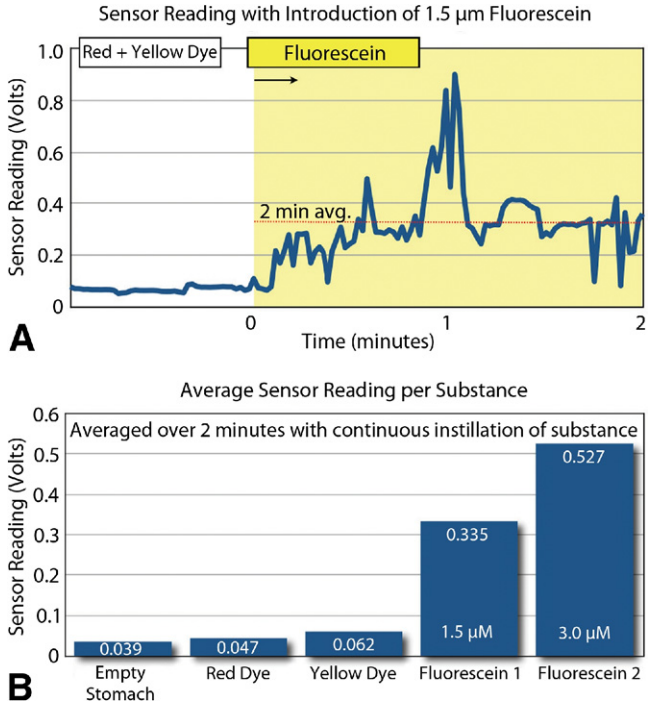

Figure 5. Benchtop data. **A**. Signal induced by blood mixed with 1.5 mol fluorescein instilled for 2 minutes. **B**. Average signal over 2 minutes induced by various substances.

surveillance of rebleeding. Second, fluorescein is relatively inexpensive and is generally considered safe because it has been in medical use for more than 100 years, most recently in endoscopic confocal microscopy.[26] Finally, fluorescein has long been used as a quantitative intravascular tracer because it is 80% bound to (Information extracted from the Fluorescein Novartis package insert) plasma albumin[27] and 10% to 15% bound to erythrocytes. Fluorescein and its metabolites also have a plasma half-life of 4 to 6 hours,[28] which, coupled with the sensitivity of our initial prototype, may allow daily intravenous dosing but will more likely require a scheduled dosing regimen. Alternatively, fluorescein could be selectively administered only when there is a clinical suspicion of bleeding.

Variations in biosensor deployment schemes are possible. For example, the biosensors can be deployed singly or in multiples because a hierarchical network can, in fact, be established to allow multiple lines of communication among biosensors and external receiver/computer. Multiple biosensors may increase sensitivity. Additionally, the biosensors could be deployed in the duodenum, a location that could theoretically allow more optimal sampling of fluids originating from the proximal upper GI tract given the funneling effect of the pylorus and the combination of the small-bowel diameter and small-bowel peristalsis. With mucosal attachment via clips and biodegradable sutures, the biosensors are designed to dislodge after days to weeks.

The main limitation of this study is that it was an acute animal study. Exact diagnostic accuracy (sensitivity and specificity) remain to be clarified in an in vivo model subjected to randomized test conditions (bleeding and nonbleeding states). However, empirically the biosensor is extremely sensitive because it was designed to detect an exogenous substance. Furthermore, in vivo functioning after 24 to 48 hours of implantation remains to be assessed.

In conclusion, an endoscopically implantable wireless biosensor for immediate detection of UGIB succeeded in a porcine bleeding model. It was able to detect a small volume of fresh blood with successful data transmission to a remote computer and cell phone. This has particular implications for the detection of rebleeding of endoscopically defined sources of GI hemorrhage when the risk of rebleeding is highest.

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APPENDIX A: BENCH-TOP DEVELOPMENT

Benchtop Testing

Ex vivo porcine studies were performed to provide initial measurements of the biosensor's sensitivity, discriminatory power, and ability to wirelessly transmit through fluid and soft tissue. As such, the benchtop study was comprised of 2 components: (1) biosensors were placed in ex vivo porcine stomachs and biosensor readings (voltage) were recorded as various substances were endoscopically instilled in the stomachs, including (a) 200 mL of red food coloring; (b) 200 mL of yellow food coloring; (c) 1.5-μmol concentration of fluorescein (AK-Fluor 10%, Akorn Inc, Lake Forest, Ill) mixed in heparinized porcine blood; (d) 3.0-μmol concentration of fluorescein mixed in heparinized porcine blood. The average reading during 2 minutes of steady instillation was recorded. Of note, 1.5 and 3.0 μmol would be approximately 2 orders of magnitude less than the 200- to 300-μmol concentration that would be achieved immediately after a standard 500-mg injection in a human adult.[20] (2) Additionally, the biosensor's capability for wireless signal transmission through fluid and gastric tissue to an external transceiver located 5 feet away was assessed.

Benchtop Results

Benchtop results of the initial biosensor prototype demonstrated a signal of 0.3 to 0.9 V for 2 minutes of continuous instillation of 1.5 μmol concentration of fluorescein in blood (Fig. 5A). Figure 5B shows the average signal reading over 2 minutes for an empty stomach, red dye, yellow dye, and 2 concentrations of fluorescein in blood. The average signal reading for fluorescein in blood was five- to 13-fold higher compared with an empty stomach, red dye, and yellow dye. Furthermore, the biosensors were able to achieve wireless data transmission through soft tissue and while submerged. Given these favorable benchtop results, it was thought appropriate to advance to acute animal labs.