

# Microneedles for Drug Delivery via the Gastrointestinal Tract

GIOVANNI TRAVERSO,<sup>1,2,3</sup> CARL M. SCHOELLHAMMER,<sup>2,3</sup> AVI SCHROEDER,<sup>3,4</sup> RUBY MAA,<sup>2</sup> GREGORY Y. LAUWERS,<sup>5</sup> BARIS E. POLAT,<sup>2,3</sup> DANIEL G. ANDERSON,<sup>2,3,6,7</sup> DANIEL BLANKSCHTEIN,<sup>2</sup> ROBERT LANGER<sup>2,3,6,7</sup>

<sup>1</sup>Division of Gastroenterology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

<sup>2</sup>Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts

<sup>3</sup>Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts

<sup>4</sup>Department of Chemical Engineering, Technion – Israel Institute of Technology, Haifa 32000, Israel

<sup>5</sup>Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

<sup>6</sup>Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

<sup>7</sup>Harvard-MIT Division of Health Science and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received 30 July 2014; revised 30 August 2014; accepted 2 September 2014

Published online 22 September 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24182

**ABSTRACT:** Both patients and physicians prefer the oral route of drug delivery. The gastrointestinal (GI) tract, though, limits the bioavailability of certain therapeutics because of its protease and bacteria-rich environment as well as general pH variability from pH 1 to 7. These extreme environments make oral delivery particularly challenging for the biologic class of therapeutics. Here, we demonstrate proof-of-concept experiments in swine that microneedle-based delivery has the capacity for improved bioavailability of a biologically active macromolecule. Moreover, we show that microneedle-containing devices can be passed and excreted from the GI tract safely. These findings strongly support the success of implementation of microneedle technology for use in the GI tract. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:362–367, 2015

**Keywords:** mucosal drug delivery; gastrointestinal; macromolecular drug delivery; oral drug delivery; membrane transport; microneedles; pill; drug delivery systems

## INTRODUCTION

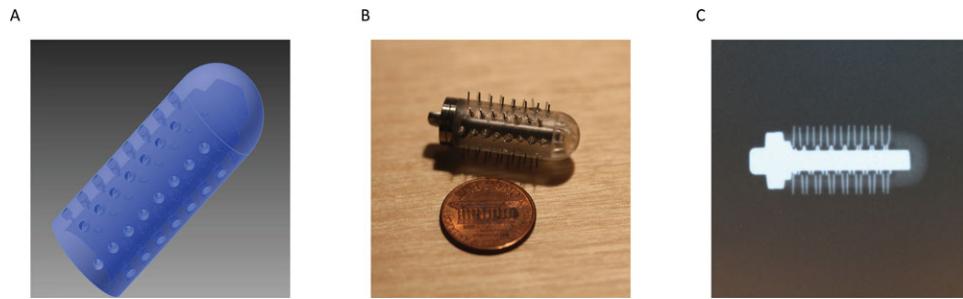
Oral drug administration remains the preferred method, particularly when compared with parenteral routes.<sup>1,2</sup> Oral drug delivery, however, is limited by poor drug absorption and drug degradation. This is of particular concern for the biologic class of drugs (such as insulin, monoclonal antibodies, and nucleic acids), which are susceptible to proteases, endonucleases, bacteria, and the extremes in pH encountered in the gastrointestinal (GI) tract.<sup>3</sup> As a result, biologics are not currently orally administrable and require delivery through injection. Several approaches have been pursued in an attempt to enable oral administration of biologics, including coadministration with enzyme inhibitors, chemical modification of the drug, polymeric micro- and nanocarriers, liposome carriers, as well as targeted nanoparticles.<sup>4–6</sup> However, these approaches require reformulation of the active pharmaceutical ingredient (API) to ensure both compatibility with the specific technique and that the activity of the API is maintained. Physical methods of administration provide an alternative means of delivery, requiring minimal reformulation of the drug, providing a potentially broad delivery platform. Similarly, these methods have the potential to deliver macromolecules. Microneedle-based technology has been extensively evaluated for transdermal drug and vaccine delivery<sup>7</sup> to many parts of the body, including the perianal skin

area for the treatment of fecal incontinence.<sup>8</sup> Unlike the skin, the GI tract is insensate and therefore provides a unique opportunity for the use of needle-based delivery systems. Moreover, the likelihood of efficacy and safety of delivery across the GI barrier with needles is supported by the extensive gastroenterological experience with GI mucosal injection as well as by the literature on the ingestion of foreign objects. Epinephrine injections in the GI tract are part of the standard of care with respect to the treatment of bleeding ulcers as well as polypectomy-induced GI bleeding.<sup>9</sup> Despite being used for localized vasoconstriction of bleeding vessels at ulcer sites, a common observation during these procedures is a near immediate tachycardic response in the patient,<sup>9</sup> supporting the systemic bioavailability of epinephrine when administered via the GI mucosa.<sup>10</sup> With regards to safety, inadvertent or purposeful ingestion of sharp and foreign objects has helped establish clinical guidelines with respect to object characteristics and object length for risk stratification of clinical complications and therefore guidance for clinical management.<sup>11</sup> Surprisingly, the overwhelming majority of foreign objects, including sharp objects, are capable of being passed via the GI tract without complications.<sup>12</sup> A large case series of 542 patients reporting the ingestion of foreign bodies noted that in those patients where foreign bodies were retained and surgical removal was required, the size range of the objects was large, approximately 3–16 cm<sup>13</sup> well above the size range of needles used in the proposed ingestible devices. Taken together, these prior observations would suggest that drug delivery may be possible from a capsule containing needles in a safe manner. Specifically, one could imagine an ingestible capsule containing radially protruding microneedles that could be used as a platform for the oral delivery of a broad range of therapeutics currently limited to injection (Fig. 1). This presents an

Correspondence to: Daniel G. Anderson (Telephone: +617-324-3634; Fax: +617-258-8827; E-mail: dgander@mit.edu); Daniel Blankschtein (Telephone: +617-253-4594; Fax: +617-252-1651; E-mail: dblank@mit.edu); Robert Langer (Telephone: +617-253-3107; Fax: +617-258-8827; E-mail: rlanger@mit.edu)

Giovanni Traverso, Carl M. Schoellhammer, and Avi Schroeder contributed equally to this work.

Journal of Pharmaceutical Sciences, Vol. 104, 362–367 (2015)  
© 2014 Wiley Periodicals, Inc. and the American Pharmacists Association



**Figure 1.** A cylindrical microneedle pill for the oral administration of biologic drugs. (a) Computer-aided design of the radial prototype housing used for *in vivo* safety evaluation. (b) Finished prototype used for *in vivo* safety showing the metal endcap and pin. (c) Radiography of the prototype in (b). Pill length 2 cm, diameter 1 cm, needle gauge: 25G.

unexplored mode of drug administration. To motivate further development of this technology, such a device would need to (1) demonstrate acceptable bioavailability comparable to that achieved through standard injection and (2) safely pass through the GI tract. To address these two critical issues, here we investigate the bioavailability of a model biologic macromolecule (insulin) via the GI tract and compare these with the kinetics achieved through traditional subcutaneous injection. We then examine the safety and feasibility of passing a model device, as well as its approximate retention time, for the purpose of guiding the design of subsequent microneedle-based GI drug delivery systems.

## MATERIALS AND METHODS

### Device Design and Construction

Computer-aided design software (Solidworks; Dassault Systems, Waltham, Massachusetts) was utilized for the design of the prototype for safety evaluation (Fig. 1a). This was fabricated from clear acrylic and 25G needles protruding 5 mm from the surface were fitted manually into the orifices. The device was 2 cm in length and 1 cm in diameter. A central metallic core was included for increasing the radio-opacity for rapid radiographic detection of the device.

### In Vivo Insulin Delivery

All procedures were conducted in accordance with protocols approved by the Massachusetts Institute of Technology Committee on Animal Care. Insulin was chosen as a model biologic because it is recognized to have negligible oral bioavailability. It also induces a rapid physiological response (reduction of blood glucose), which can be readily monitored and quantified in real time. *In vivo* porcine studies were performed on three Yorkshire pigs weighing approximately 75–80 kg. Prior to the procedures, the animals were fasted overnight. On the day of the procedure, the morning feed was withheld and the animal was sedated. Following induction of anesthesia with intramuscular injection of Telazol (tiltamine/zolazepam) 5 mg/kg, xylazine 2 mg/kg, and atropine 0.04 mg/kg, the pigs were intubated and maintained on isoflurane (1%–3% inhaled). After sedation, a catheter was placed in the femoral vein using the Seldinger technique to allow for frequent blood sampling. Prior to administration of insulin, 4 mL blood samples were taken from the catheter in the femoral vein to quantify the animal's starting blood-glucose levels. A real-time readout was achieved using a TRUEtrack® blood glucose meter (Nipro Diagnostics Inc., Fort

Lauderdale, Florida) and the remainder of the blood sample was saved in a blood collection tube with sodium fluoride and ethylenediaminetetraacetic acid to minimize further glucose metabolism (Beckton Dickinson, Franklin Lakes, New Jersey). All data shown represent the blood-glucose values quantified from the blood collection tubes.

Following baseline blood collections to establish an initial blood-glucose level, 10 units of rapid acting insulin aspart (NovoLog; Novonordisk, Bagsværd, Denmark) in 1 ml of 0.9% saline was administered using a 25G Carr-Locke Needle (US Endoscopy, Mentor, Ohio). Injections were performed in triplicate on separate experimental days in the stomach, duodenum, colon, and skin. A submucosal injection was confirmed via direct endoscopic visualization of a submucosal expansion. Colonic injection was preceded by a tap water enema to facilitate tissue visualization. Subcutaneous injections were performed using a 25G needle in the anterior abdominal wall of the animal. It should be noted that only one injection in one tissue area was administered to an animal on a given day. Upon injection, blood was sampled from the catheter approximately every 2 min and analyzed as described above. The animal's blood-glucose was monitored in this way until no further drop occurred or until a blood-glucose concentration of 40 mg/dL was achieved in order not to harm the animal. Persistent hypoglycemia under 40 mg/dL was corrected with intravenous boluses of 50% dextrose. Blood-glucose values presented are normalized by the animal's initial value, defined as the last blood-glucose value observed before injection of insulin.

### Evaluation of Device Passage and Safety Assessment

To place the prototype shown in Figure 1b, the animal was first sedated and intubated as described above. Then, an overtube (US Endoscopy) was placed in the esophagus. The microneedle pill was deployed in the stomach under direct endoscopic visualization. Placement was further confirmed radiographically. Animals were evaluated clinically twice daily for any evidence of obstruction including abdominal distension, lack of fecal material in the cage, and vomiting while evidence of the device remained radiographically visible. Radiographs were performed every 48–72 h. The retention time of the device was estimated based on when it was no longer visible on radiographs. Post-mortem inspection of the entire GI tract confirmed passage of the device. The GI tissue was evaluated for any macroscopic evidence of damage. Furthermore, sections were taken from the pylorus, ileocecal valve, and anal canal, representing the three points of constriction distal to the stomach, and evaluated for

any evidence of macroscopic and microscopic damage through analysis of hematoxylin and eosin-stained tissue sections.

### Statistical Analysis

The time necessary to observe a drop in the animal's blood-glucose as a result of insulin administration was defined as a drop in initial blood-glucose  $\geq 5\%$ . Insulin administration in each tissue was repeated on separate days at least three times.

Statistical significance was assessed by one-way ANOVA. Statistical significance is defined as  $p < 0.05$ . All calculations were performed using MatLab R2014a (MathWorks, Natick, Massachusetts).

## RESULTS

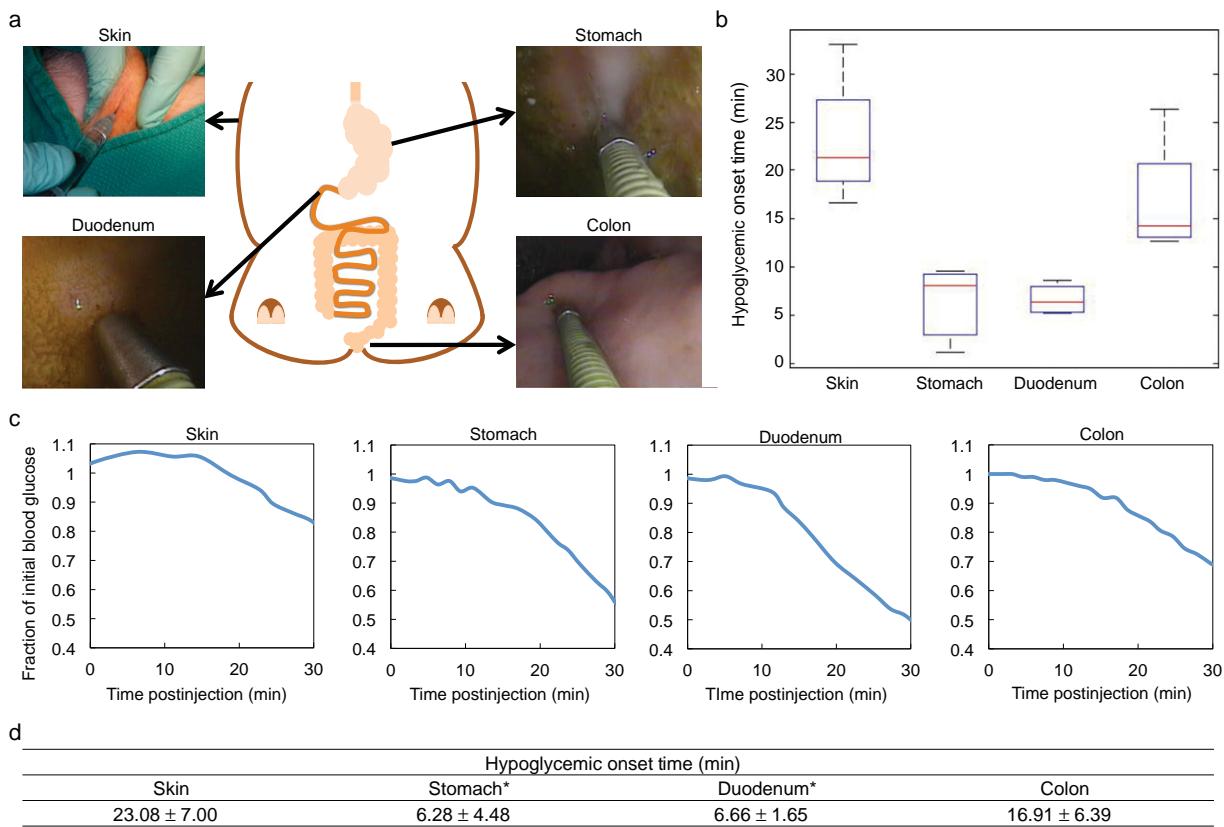
### Systemic Delivery of Insulin

The ability for systemic delivery of insulin was evaluated through serial injections via the gastric, duodenal, and colonic mucosa of approximately 75–80 kg Yorkshire pigs. Subcutaneous insulin administration was used as a comparator (Fig. 2a). The induction of hypoglycemia was monitored following the administration of insulin, and the time to hypo-

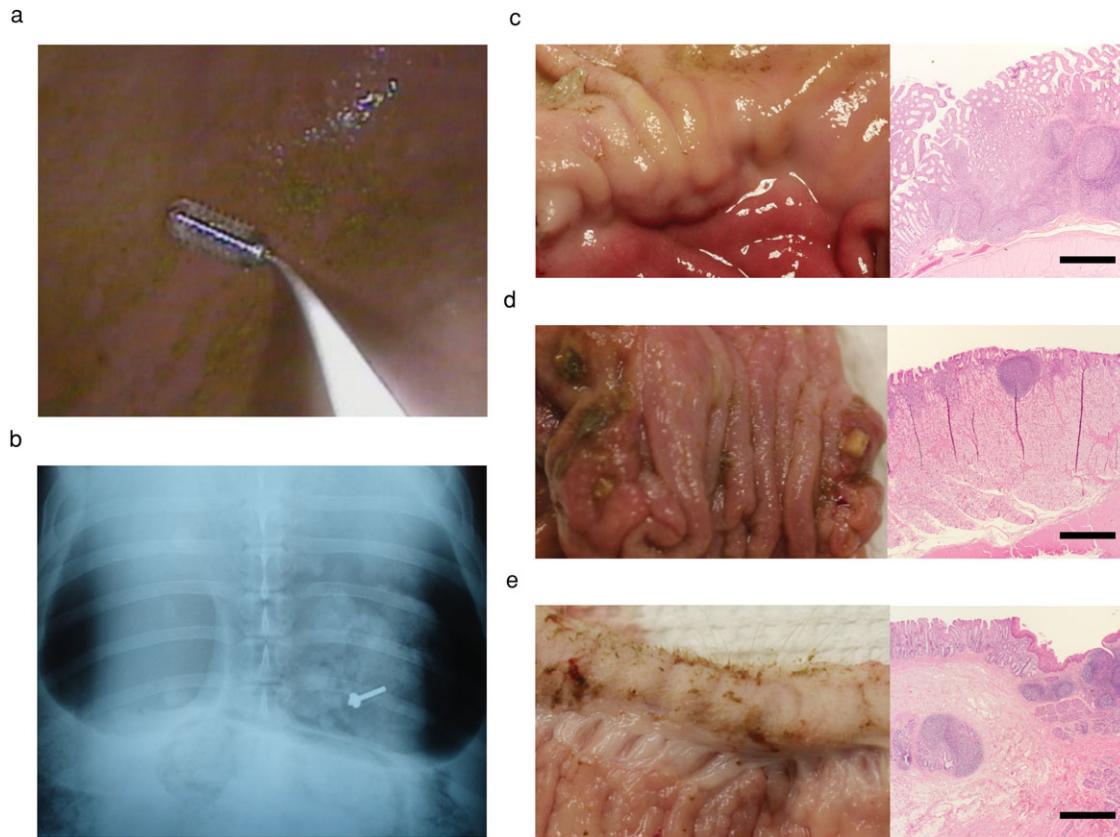
glycemic onset (defined as a drop in the initial blood-glucose  $\geq 5\%$ ) was used for comparison across the varying anatomic sites (Figs. 2b–2d). Hypoglycemic onset following the injection of 10 units of rapid acting insulin was observed at  $23.08 \pm 7.00$ ,  $6.28 \pm 4.48$ ,  $6.66 \pm 1.65$ , and  $16.91 \pm 6.39$  min for subcutaneous, gastric, duodenal, and colonic administration, respectively. The onset time was significantly diminished when insulin was administered via the GI tract as compared with traditional subcutaneous administration (Figs. 2b–2d). Although the average onset time via the colon was shorter than that observed via the skin, the difference was not statistically significant. However, administration via the gastric and duodenal mucosa demonstrated a significant reduction in the onset time compared with subcutaneous administration ( $p < 0.008$ ).

### Safety Evaluation of a Microneedle Prototype in the GI Tract

The safety and ability for natural passage of a microneedle-containing device via the GI tract was investigated. Safety and passage time was estimated using the custom-built device shown in Figure 1b. The dimensions of this prototype were modeled around those of US FDA-approved ingestible devices, such as the video capsule endoscope.<sup>14</sup> The microneedles were placed radially around the device to ensure maximal apposition



**Figure 2.** (a) Images of insulin injection in three different regions of the GI tract compared with subcutaneous administration. Clockwise from upper left: skin, stomach, colon, and duodenum. Representative images of the injections are shown. (b) Time in minutes to observe a drop in blood-glucose as a result of injection of insulin in the various GI tissue and skin. The median, 25th, and 75th percentiles are given. The whiskers indicate the most extreme data points. (c) Representative plots of normalized blood-glucose with time as a result of insulin injection subcutaneously, or through the stomach, duodenum, or colon. (d) Time in minutes to observe a drop in blood-glucose as a result of injection of insulin in the various GI tissue and skin. Averages and standard deviations are given. \*Statistical significance compared with skin based on a multiple comparisons test from ANOVA ( $p < 0.008$ ).



**Figure 3.** Safety assessment surrounding passage of the microneedle pill. (a) Endoscopic deployment of the device in the stomach. (b) X-rays are taken to monitor the progression of the pill. Representative gross and histological images of the (c) pylorus, (d) ileocecal valve, and (e) anal canal after natural passage of the device. The scale bar in the histology images represents 1 mm.

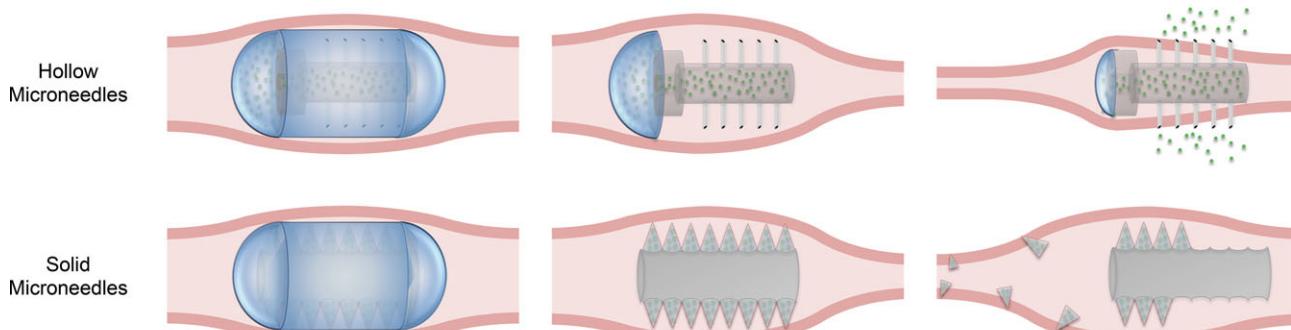
of the needles with the GI mucosa. A metal core was added to aid in the visualization of the pill on radiographs (Fig. 1c).

The device was endoscopically deployed in the stomach of three animals as shown in Figure 3a. Animals were monitored daily and radiographs were taken to track the pill movement and to monitor for any evidence of intestinal obstruction or perforation (Fig. 3b). Throughout the transit time of the prototype, all animals remained free of clinical signs of obstruction. Furthermore, radiographs remained free of evidence of intestinal obstruction or perforation. Loss of a detectable radiopaque device on the radiographs was used to determine the approximate transit time of the prototypes. The passage time of the device in three different animals was 7, 19, and 56 days. Upon loss of the radiopaque device, animals were euthanized, and the entire GI tract was examined and found to be macroscopically normal. Further, the three sites of constriction in the GI tract distal to the site of prototype deployment (pylorus, ileocecal valve, and anal canal) were examined and appeared normal (Figs. 3c–3e). Additionally, these three points were also fixed in formalin for histological examination (Figs. 3c–3e). Histological examination was notable for normal appearing tissue at all three sites of constriction in the GI tract in the three animals.

## DISCUSSION

Here, we report *in vivo* proof-of-concept experiments supporting the feasibility and safety of microneedle-based trans-GI

delivery of a macromolecule. With regards to bioavailability, delivery was found to be more effective than subcutaneous administration. Specifically, GI-based delivery afforded improved pharmacokinetics and a more robust hypoglycemic effect. Because of the extensive investigation into the use of microneedles for transdermal delivery, we compared these results to recent literature reports of microneedle-based transdermal delivery of insulin.<sup>15–17</sup> To compare delivery routes, the subcutaneous administration of insulin included in each study was used as a control. Then, the efficacy of each route relative to its respective subcutaneous control was assessed and compared qualitatively. This was carried out to account for differences in experimental methods across studies. In the transdermal studies, subcutaneous administration always afforded a faster hypoglycemic onset time compared with microneedle-based transdermal delivery of insulin. Even when multiple techniques were employed transdermally (microneedles and iontophoresis), subcutaneous administration afforded a faster onset time. This should be contrasted with the findings presented here, where microneedle-based trans-GI delivery affords faster onset compared with subcutaneous injection. Not having to use multiple techniques also greatly simplifies administration.<sup>15–17</sup> Although the bioavailability presented here is likely to be higher than that from a fully integrated microneedle capsule, it was critical to first demonstrate the bioavailability of therapeutics administered in this way to confirm this mode of administration as adequate for the delivery of macromolecules. There are many possible avenues of exploration surrounding the bioavailability achieved



**Figure 4.** Therapeutic use concept of the microneedle pill. Both hollow and solid microneedles could be used. In both cases, the pill's needles are initially coated by a pH-responsive coating to aid in ingestion (left). When the pill has reached the desired location in the GI tract, the coating dissolves, revealing the microneedles (middle). In the case of hollow microneedles (top right), the drug reservoir is compressed through peristalsis, releasing the drug through the needles. In the case of solid microneedles (bottom right), the drug is formulated into the microneedles. The microneedles penetrate the tissue and break off of the pill, leaving the needle to release the drug in a controlled manner, based on the needle formulation.

from a stand-alone device. For example, solid, drug-containing microneedles could be fabricated from biocompatible polymers. These could then detach from the capsule and become lodged in the GI tissue, where they could slowly release their payload (Fig. 4). Additionally, the peristaltic motion in the GI tract could be utilized to compress the capsule, leading to release of drug only when the tissue is in immediate contact with the needles. Evaluation of the drug release kinetics and the kinetics of clearance of the device as a result of varying microneedle geometries will be required to fully characterize future iterations of devices supported by this work.

In addition to the bioavailability of compounds administered in this way, the safety and natural passage of such a device are paramount to further investigation. To this end, safety evaluation in the swine model confirmed the absence of any intestinal obstruction or GI mucosal damage and demonstrated the ability to pass such a device. Additionally, histological examination was notable for normal GI mucosa at the three distal points of constriction, which are at greatest risk for damage. It should be noted that 25 gauge needles were purposefully used for the device shown in Figure 1b. These needles have an outer diameter exceeding 500  $\mu\text{m}$ , increasing the likelihood of perforation. The safe passage of this particular prototype, therefore, is reassuring and further indicative of the potential for this new method of macromolecule delivery to be safe.

With respect to retention time in our experiments, our device was retained for a minimum of 7 days. Typical GI retention times in pigs have previously been reported to range from 2 to 33 days.<sup>18,19</sup> The large range in observed retention times of the device in this study may be attributed to the increased interaction between the microneedles and the GI mucosa but may also reflect the transient gastric retention of the device. Specifically, the retention time of objects in the stomach of pigs has previously been estimated to range from 1 to 28 days.<sup>18,19</sup> This could be because of the quadrupedal nature. It has also been noted that gastric retention scales with object size.<sup>20</sup> As a result, the geometry and design of the microneedles would be another interesting area of investigation for their effect on retention time. The safety and tolerability of this device over an extended period of time, however, is encouraging and raises the possibility of using derivatives

of this device for extended-release oral formulations of both small molecule therapeutics as well as biologics with once a week dosing based on the minimum retention time of 7 days that we observed. Further elucidation of the parameters determining consistent retention at varying time points will help develop future extended drug release systems. Taken together, this work will serve as the catalyst for a significant change in the development of oral delivery systems for macromolecules enabling the bypassing of the harsh GI mucosal environment.

## CONCLUSIONS

The oral route of drug administration is the most convenient route for patients.<sup>1,2</sup> However, the hostile environment present in the GI tract limits oral delivery to small molecules. As a result, the biologic class of drugs is mainly limited to needle-based administration. Physical delivery methods, such as microneedles, might enable a platform technology for the oral delivery of a broad range of substances. To this end, here we present a proof-of-concept study involving the use of microneedles for the delivery of biologics via the GI tract for the first time. The blood-glucose response kinetics of a model macromolecule, insulin, was significantly improved compared with the subcutaneous route when administered via the GI tract, demonstrating that the bioavailability of a model compound is still sufficient when administered via injection in the GI tract. To investigate the potential tolerability of such a device, results surrounding the safe passage of such a device were presented. Specifically, a model device having exposed radially protruding microneedles was safely passed via the GI tract without any evidence of tissue damage. With additional investigation, we anticipate multiple variations of devices enabling the oral administration of therapeutics from capsules containing microneedles. These include utilizing the peristaltic motion of the GI tract to stimulate microinjection using hollow microneedles, or needle dislodgement where the needles are fabricated from drug-loaded polymers (Fig. 4). By demonstrating the potential safety and efficacy of this method, this study provides the basis for further development of integrated microneedle devices for oral macromolecule delivery.

## ACKNOWLEDGMENTS

This work was funded in part by NIH grant EB000244 (to R.L.), NIH grant T32DK7191-38-S1 (to G.T.), and NIH grant CA014051 for the Hope Babette Tang (1983) Histology Facility. We would like to thank Pentax for providing the endoscopic equipment used for this research, and in particular Mr. Mike Fina for facilitating access to the equipment. We also thank Monica Siddalls and Robert Marini for their excellent veterinary support as well as Andrew Ryan from the MIT Central Machine Shop for his assistance in assembling prototypes. We dedicate this paper to the memory of Officer Sean Collier, for his caring service to the MIT community and for his sacrifice.

The authors declare Provisional US patent application 13/728,300 filed on December 27, 2012.

## REFERENCES

1. Mignani S, Kazzouli El S, Bousmina M, Majoral J-P. 2013. Expand classical drug administration ways by emerging routes using dendrimer drug delivery systems: A concise overview. *Adv Drug Deliv Rev* 65(10):1316–1330.
2. Borner MM, Schoffski P, de Wit R, Caponigro F, Comella G, Sulkes A, Greim G, Peters GJ, van der Born K, Wanders J, de Boer RF, Martin C, Fumoleau P. 2002. Patient preference and pharmacokinetics of oral modulated UFT versus intravenous fluorouracil and leucovorin: A randomised crossover trial in advanced colorectal cancer. *Eur J Cancer* 38(3):349–358.
3. Aoki Y, Morishita M, Asai K, Akikusa B, Hosoda S, Takayama K. 2005. Region-dependent role of the mucous/glycocalyx layers in insulin permeation across rat small intestinal membrane. *Pharm Res* 22(11):1854–1862.
4. Chaturvedi K, Ganguly K, Nadagouda MN, Aminabhavi TM. 2013. Polymeric hydrogels for oral insulin delivery. *J Control Release* 165(2):129–138.
5. Radwant MA, Aboul-Enein HY. 2002. The effect of oral absorption enhancers on the in vivo performance of insulin-loaded poly(ethylcyanoacrylate) nanospheres in diabetic rats. *J Microencapsul* 19(2):225–235.
6. Jain S, Rathi VV, Jain AK, Das M, Godugu C. 2012. Folate-decorated PLGA nanoparticles as a rationally designed vehicle for the oral delivery of insulin. *Nanomedicine (Lond)* 7(9):1311–1337.
7. Kim Y-C, Park J-H, Prausnitz MR. Microneedles for drug and vaccine delivery. 2012. *Adv Drug Deliv Rev* 64(14):1547–1568.
8. Baek C, Han M, Min J, Prausnitz MR, Park J-H, Park J. 2011. Local transdermal delivery of phenylephrine to the anal sphincter muscle using microneedles. *J Control Release* 154:138–147.
9. Lau JYW, Sydney Chung SC. 2010. Hemostasis: Injection Sclerotherapy, Banding, Mechanical Methods, Heater Probe and Other Methods. In Classen M, Tytgat GNJ, Lightdale CJ, editors. *Gastroenterological Endoscopy*, 2nd ed., Stuttgart: Thieme Publishers Co. p262.
10. Frida A, Bashar A, Srikanth P. 2000. Ventricular tachycardia after endoscopic injection of epinephrine for bleeding ulcer. *Am J Gastroenterol* 95:2618.
11. Ikenberry SO, Jue TL, Anderson MA, Appalaneni V, Banerjee S, Ben-Menachem T, Decker GA, Fanelli RD, Fisher LR, Fukami N, Harrison ME, Jain R, Khan KM, Krinsky ML, Maple JT, Sharaf R, Strohmeyer L, Dominitz JA. 2011. Management of ingested foreign bodies and food impactions. *Gastrointest Endosc* 73(6):1085–1091.
12. Butterworth JR, Wright K, Boulton RA, Pathmakanthan S, Goh J. 2004. Management of swallowed razor blades—Retrieve or wait and see? *Gut* 53(4):477.
13. Velitchkov NG, Grigorov GI, Losanoff JE, Kjossev KT. 1996. Ingested foreign bodies of the gastrointestinal tract: Retrospective analysis of 542 cases. *World J Surg* 20(8):1001–1005.
14. Iddan G, Meron G, Glukhovsky A, Swain P. 2000. Wireless capsule endoscopy. *Nature* 405(6785):417.
15. Liu S, Jin M-N, Quan Y-S, Kamiyama F, Katsumi H, Sakane T, Yamamoto A. 2012. The development and characteristics of novel microneedle arrays fabricated from hyaluronic acid, and their application in the transdermal delivery of insulin. *J Control Release* 161(3):933–941.
16. Zhou C-P, Liu Y-L, Wang H-L, Zhang P-X, Zhang J-L. 2010. Transdermal delivery of insulin using microneedle rollers in vivo. *Int J Pharm* 392(1–2):127–133.
17. Chen H, Zhu H, Zheng J, Mou D, Wan J, Zhang J, Shi T, Zhao Y, Xu H, Yang X. 2009. Iontophoresis-driven penetration of nanovesicles through microneedle-induced skin microchannels for enhancing transdermal delivery of insulin. *J Control Release* 139(1):63–72.
18. Snoeck V, Huyghebaert N, Cox E, Vermeire A, Saunders J, Remon JP, Verschooten F, Goddeeris BM. 2004. Gastrointestinal transit time of nondisintegrating radio-opaque pellets in suckling and recently weaned piglets. *J Control Release* 94(1):143–153.
19. Hossain M, Abramowitz W, Watrous BJ, Szpunar GJ, Ayres JW. 1990. Gastrointestinal transit of nondisintegrating, nonerodible oral dosage forms in pigs. *Pharm Res* 7(11):1163–1166.
20. Aoyagi N, Ogata H, Kaniwa N, Uchiyama M, Yasuda Y, Tanioka Y. 1992. Gastric emptying of tablets and granules in humans, dogs, pigs, and stomach-emptying-controlled rabbits. *J Pharm Sci* 81(12):1170–1174.