A microneedle platform for buccal macromolecule delivery

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Alternative means for drug delivery are needed to facilitate drug adherence and administration. Microneedles (MNs) have been previously investigated transdermally for drug delivery. To date, drug loading into MNs has been limited by drug solubility in the polymeric blend. We designed a highly drug-loaded MN patch to deliver macromolecules and applied it to the buccal area, which allows for faster delivery than the skin. We successfully delivered 1-mg payloads of human insulin and human growth hormone to the buccal cavity of swine within 30 s. In addition, we conducted a trial in 100 healthy volunteers to assess potential discomfort associated with MNs when applied in the oral cavity, identifying the hard palate as the preferred application site. We envisage that MN patches applied on buccal surfaces could increase medication adherence and facilitate the painless delivery of biologics and other drugs to many, especially for the pediatric and elderly populations.

INTRODUCTION

Dosing of biologics is generally limited to parenteral routes, which are associated with fear and discomfort, particularly in the pediatric population, thereby potentially affecting adherence (1). Biologics have transformed our capacity to effectively treat a range of conditions including diabetes mellitus, growth hormone insufficiency, and enzyme deficiencies, which require protein- and nucleic acid–based therapies (2–4). However, biologics are generally degraded in the gastrointestinal tract or poorly absorbed (5, 6). Therefore, injections have been the standard mode of administration of these therapeutics. Injections are associated with fear and discomfort, needle stick injury, risk of infection, and potential injury to local tissues, which is especially problematic in the pediatric population, thereby affecting adherence (7–10). In addition to being a major stressor for some adults and children, injections can lead to permanent nerve injury, muscle fibrosis, and vascular damage with detrimental outcomes in premature or small infants (11). Thus, there is a need for alternative drug delivery modes and routes (12–14).

Various transdermal drug delivery systems including microand millineedles, ultrasound, microjets, and iontophoresis have been developed and, in some instances, have reached clinical evaluation (12, 15). In parallel, several nonphysical or enhancer- and formulation-based approaches for improving transdermal and gastrointestinal permeability have made it to the clinic and obtained U.S. Food and Drug Administration approval (16–19). Of the existing delivery systems, we identified microneedle (MN) patches as a promising candidate to meet the needs of vulnerable populations, such as pediatric and elderly patients, receiving biologic therapy. MN patches provide a solid matrix with the potential for high drug loading, structural integrity to facilitate penetrative administration, and material fabrication options to enable maximal in-tissue dissolution and delivery. To allow for delivery of high drug loads and overcome the need to have patches remain in place, we identified the oral or buccal mucosa as a potential site for MN-based delivery. While the skin remains the main target for MN application due to accessibility, the buccal cavity—despite its curved nature and moist surface—is gaining focus for MN delivery (20, 21).

In this study, we deliver macromolecules including human insulin (HI) and human growth hormone (hGH) using MNs to the buccal mucosa. Its accessibility through the mouth and the lack of stratum corneum, together with minimal scarring and faster relative wound healing, make the buccal mucosa an attractive site to deliver drugs, especially to the pediatric population (22). Additional advantages of the buccal mucosa include its neutral pH, the absence of the hepatic and intestinal first-pass metabolism, and the presence of fewer digestive enzymes.

Dissolving MNs usually contain the active pharmaceutical ingredient (API) or drugs mixed within a biocompatible polymeric matrix, which dissolves when in contact with tissue to deliver the payload (23). The amount of payload in dissolving MNs is often limited by the size and solubility of the API within the polymeric matrix. Here, we describe a novel fabrication technique to produce clinically relevant highly concentrated MNs capable of delivering...
HI and hGH to the buccal mucosa. We aimed to develop a rapid drug deposition system into the buccal mucosa that exhibited easy patch placement and was compatible with different macromolecular drugs (Fig. 1A). To evaluate the feasibility and acceptability of this technology, we investigated the potential discomfort and pain associated with our MN patch on 100 human volunteers using a visual analog pain scale score. Through the clinical trial, we evaluated a range of buccal sites by obtaining subject feedback on their preferred buccal sites of administration and their preferences relative to standard transdermal hypodermic needle administration.

In summary, our MN patches are capable of systemically, quickly, and painlessly delivering relevant macromolecular drug doses via insertion to the buccal mucosa. The painless sensation reported by the study participants suggests that our MN patch would potentially facilitate drug delivery to the pediatric population.

RESULTS

Highly loaded MN patches

By loading the API in the solid form to the MN molds, we successfully prepared sharp, highly loaded MN patches (Fig. 1B to F). This fabrication method allows the loading of 2 mg of drug on a 10 mm by 10 mm patch, corresponding to approximately 60% loading efficiency in the MNs, with the remaining 40% in the baseplate. The most effective loading was obtained when 1.5 mg of the API was placed into the mold, which resulted in 1.0 ± 0.2 mg of HI (60%) localized in the MN tips (fig. S1). To characterize the distribution of the API in the MNs, we loaded fluorescein isothiocyanate (FITC)-dextran conjugates of 3 to 5 kDa and 22 kDa, simulating HI and hGH, respectively, and evaluated these with confocal microscopy, confirming that API preferentially settles into the MN tips (Fig. 1B and fig. S2).

Penetration force and dissolution of MNs

We characterized the insertion force required to insert single hypodermic needles (“penetration force”) of varying gauges (30.5, 25, and 18 gauge) into human tissues (where available) and swine buccal tissue. As expected, as the diameter of the needle increased, the penetration force increased (fig. S3). This relationship is true for both swine and human tissues. Swine tissue penetration forces were consistently higher than those of humans for the same anatomical location. For example, the penetration force of 30.5-, 25-, and 18-gauge needles applied to the cheek were (0.177 N, 0.094 N), (0.252 N, 0.199 N), and (0.782 N, 0.417 N) for swine and human, respectively. The same pattern holds true for skin, tongue, and sublingual sites. In swine, the penetration force was highest in the palate when using 30.5- and 25-gauge needles, followed by skin, lip, cheek, sublingual, and tongue. In human tissue, the highest penetration force was recorded in skin for all gauges, followed by tongue, cheek, and sublingual. Note that human lip and human palate samples were not available and thus could not be tested in this analysis.

The high API concentration in the MN tips did not compromise the MN sharpness as depicted in Fig. 1 (D to F). To ensure that these results correlated with the in vivo application of MNs, further studies of MN penetration and dissolution were performed to validate the integrity of the MNs when inserted into the tissue.

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Fig. 1. Buccal MN patch concept and fabrication. (A) Concept for the application of buccal MN patches (i) demonstrates application, (ii) MN patch, (iii) high API density in MN tips, (iv) penetration of surface of buccal mucosa, (v) in the vicinity of microvasculature, (vi) with subsequent API release and systemic absorption. (B) Confocal image of an MN array loaded with FITC-dextran (3 to 5 kDa). (C) MNs are prepared by adding approximately 1 mg of the API (HI or hGH) in powder form to the MN cavities (i) and then using a binding solution (ii) to give mechanical structure to the MN patch (iii). After drying, the MNs are demolded and ready to use (iv). (D) SEM image of an MN. (E) Optical image of MNs loaded with approximately 1 mg of HI and (F) hGH (scale bar, 500 μm).
Penetration and dissolution of the HI- and Texas Red–loaded MNs were evaluated in vivo in the oral cavity of swine. Throughout the oral cavity, dissolution of 50%, as measured by the MN height, was observed within 5 to 30 s after MN application (Fig. 2A and fig. S4). API delivery to the buccal tissue was evaluated by means of fluorescence quantitation of Texas Red–loaded MNs in vivo in swine and ex vivo in human tissues (Fig. 2, B and C, and fig. S5). Using fluorescence imaging, we observed rapid delivery of Texas Red (within 5 to 15 s) for MNs that were actively pushed into the tissue as compared with the controls, which were placed onto the tissue without any applied force. Tissue penetration of the inserted MNs was confirmed histologically (Fig. 2D and fig. S6) and by optical coherence tomography (OCT) (fig. S7).

**Drug delivery in the buccal cavity**

Various locations in the swine’s buccal cavity were tested for drug delivery. The buccal mucosa, the tongue’s dorsum surface (hereafter named “tongue”), and the tongue’s ventral surface (hereafter referred to as “sublingual”) of swine resemble the human anatomy; however, the palate of swine differs substantially from that of humans. While the hard palate in humans is arched and smooth, swine have prominent palatal ridges, which hindered the application of our 10 mm–by–10 mm MN patches. Upon insertion, the baseplates of MN patches were observed to break when applied to the ridged area, but this did not compromise the insertion of the MNs. A larger amount of macromolecular delivery was observed when the MNs were inserted onto the buccal mucosa and palate in comparison to the upper surface of the tongue or the sublingual space (Fig. 3, A to C). We therefore selected the buccal mucosa and the soft palate for further evaluation of MN application in the buccal cavity of swine.

The MNs successfully delivered HI and hGH when applied to the palate and buccal mucosa of swine (Fig. 3). The concentration of HI delivered was quantified using an AlphaLISA immunoassay, specific for HI. MN application to the palate and buccal mucosa delivered a similar amount of HI as subcutaneous injection consisting of MN tips dissolved in saline. MN application also delayed Tmax; for subcutaneous injection, Tmax was observed after 10 min, while for MN application, Tmax was observed after 40 to 50 min (Fig. 3C). HI Cmax of 76 and 135 pM were measured for polyvinylpyrrolidone (PVP) patches applied to the palate and buccal mucosa, respectively. HI concentration during the first hour after subcutaneous administration varied between 134 and 174 pM. Sorbitol MNs demonstrated higher variability when administered subcutaneously, with an HI concentration of up to 600 pM. When sorbitol-based MNs were applied to the palate, the average Cmax (130 pM) was reached after 40 min; for the buccal mucosa, the average Cmax (111 pM) was observed 70 min after application (Fig. 3D). The delivery of HI from the MNs generally resulted in a clear glucose drop (Fig. 3E). Higher HI plasma levels were observed when using PVP-based MNs, which is likely due to the recognized effects of PVP on solubility and permeability (24). In addition, from the stability studies, it can be
concluded that higher HI was recovered from the PVP patches compared with sorbitol after the different storage periods (Fig. 4B). The high temperature of the melted sorbitol may have an effect on the API properties, which could justify the differences observed in the delivery levels. Differences in the plasma levels observed with the subcutaneous injection could be attributed to the filtration step, where the PVP solution may have trapped some HI, reducing its permeation through the filter membrane and, thus, affecting the dose administered. hGH-loaded PVP MNs were able to deliver maximum average concentrations of 0.52 and 1.10 ng/ml for the palate and buccal mucosa, respectively (Fig. 3, F and G). An average peak concentration of 0.55 ng/ml was observed for sorbitol-based MNs inserted in buccal mucosa (Fig. 3H). Unlike HI, hGH levels were significantly higher for subcutaneous delivery as opposed to MN patch application, reaching a peak concentration of 120 ng/ml.

Stability of MNs under different temperature and humidity conditions

PVP and sorbitol were evaluated in parallel as binders to determine whether one formulation demonstrated superiority in the mechanical and stability tests, but overall, both had comparable outcomes. HI-loaded MNs prepared with sorbitol showed similar stability to those fabricated with PVP at the different temperatures tested, 5°C, 25°C, and 40°C, the last with a relative humidity (RH) of 75% (Fig. 4A). The addition of trehalose to the sorbitol formulation decreased the formation of covalent dimers, and this reduction was significant for the samples stored at 40°C and 75% RH. Dimer formation in the samples stored at 5°C for 3 months was below 1%.

Up to 80% of the initial HI loaded into the MNs was recovered from PVP MNs after 1 month of storage at 25°C, corresponding to approximately 1 mg of HI (Fig. 4B). The decrease in the HI recovery is partially due to the formation of the covalent dimers, the limited HI solubility in aqueous solutions, and the preparation steps (i.e., centrifugation/filtration) for quantification using chromatography. Although hGH was successfully delivered using PVP MNs, we observed higher hGH dimer formation in PVP than sorbitol (Fig. 4C) and declining purity over time (Fig. 4E), which suggests that oxidation of hGH occurred in the PVP MNs. When sorbitol was used as the matrix for the MNs, the dimer formation was under 1% after 3 months of storage at 5 and 25°C. Both PVP- and sorbitol-based MNs loaded with hGH exhibited dimer formation during all the storage conditions and lengths, with a dimerization content above 2% when stored at 40°C and 75% RH. While the addition of trehalose is known to contribute to protein stability (25, 26), as it did for HI (Fig. 4A), it did not have a significant effect on the stability of hGH when stored at a higher temperature (Fig. 4, C to E).
Human acceptability evaluation of MN buccal application

To assess patient comfort of MNs applied to the buccal cavity, we conducted an initial human acceptability study with sterilized MNs (fig. S8). Sorbitol was selected to fabricate the patches of this study for being a sugar widely used in daily consumed products. To control the application force of the patches across subjects and anatomic sites, we generated a custom-made electric applicator (fig. S9). Controlled force and speed are essential, especially when applying MNs in a highly moist environment like the buccal cavity, to ensure that the MNs have the ability to penetrate the tissue before their dissolution (25). Our applicator was programmed to reproducibly apply 10 N, providing administrator feedback via a lighting system, and enabled penetration in all tissues without causing any bleeding. Lack of bleeding was confirmed in vivo in swine when the maximum force of the applicator (20 N) was applied (Fig. 5A). The insertion sites in swine’s buccal cavity could not be seen 20 min after MN application, suggesting that the MNs had closed after their removal. In the test to validate the applicator, the desired force of 10 N could be achieved on all tissue locations in less than 2 s and in most of the sites within 1 s (Fig. 5B). Furthermore, penetration of sorbitol MNs in ex vivo human buccal tissue was confirmed using OCT (fig. S10).

This is the first study of its kind to test the perception of MN application in the buccal cavity of 100 volunteers (fig. S11). Previous studies have shown that MNs inserted into the skin are significantly less painful than hypodermic needles (27). In this study, we did not use hypodermic needles as a positive control as they may have hindered discerning the pain perception at the different locations given hypodermic needles would likely yield higher scores on the visual analog scale (VAS) than microneedle patches and could have overwhelmed the signal from the separate locations (28). The absence of this control may have manifested in higher scores on the VAS for microneedle patches. The palate was reported by the volunteers to be the preferred site of patch application, followed closely by the cheek (Fig. 5C). This was also reflected by the VAS scores reported: 1.2 ± 1.2 for palate, 1.7 ± 1.4 for cheek, 2.6 ± 2.0 for lip, 1.3 ± 1.3 for tongue, and 3.4 ± 2.2 for sublingual MN applications. These can be compared with the reported VAS score of 1.2 ± 1.0 for forearm application (Fig. 5D). Even though the participants of the study were blinded to the presence of MNs in the patches applied for all application sites, the mean reported VAS score was larger for patches with MNs than the control without MNs. MN application to the tongue proved difficult, as the patches occasionally slid on the soft, moist surface. The sublingual area was reported by the participants to be the least desirable site of application. As part of the study, 95% of subjects reported that they would choose this technology over conventional injections.

DISCUSSION

Successful drug delivery using MNs depends, among other factors, on the MN penetration depth into the tissue, which is, in turn, determined by the application force. MN penetration depth in the
skin has been reported to vary between 10 and 80% depending on numerous factors including application force, application velocity, MN shape, and MN length (29, 30). The tensile strength and thickness of the tissue, as well as the dimensions of the needles, influence the needle penetration force. Skin has a stratum corneum, which also requires a higher penetration force, especially as the needle diameter is increased. Meanwhile, the palate does not contain much soft tissue; therefore, higher penetration forces are needed to compress the thin soft tissue against the bone. The soft tissues of the buccal space are highly compliant and elastic. We have established a correlation between needle diameter and the force needed to penetrate the different tissues. Overall, we can anticipate that MNs that penetrate swine tissue will likely penetrate human tissue given the lower tissue penetration forces of the latter. Compared with skin, the low penetration forces and fast drug delivery profile in the buccal cavity present an attractive alternative means of delivering drugs to both the elderly and pediatric populations.

To date, biodegradable polymeric MNs used for drug delivery contain little API loading—often below 10% in weight—due to the API’s limited solubility in the polymeric matrix and low surface area available for coating (31, 32). Here, we present an alternative preparation method for MNs, capable of loading high amounts of API in the MN tips. Thus, these 1-cm² MN patches are able to deliver large doses of API, up to 2 mg of drug. The increased API load of our patches would potentially enable the delivery of (i) APIs in the reduced patch size and (ii) APIs that require high doses to have a relevant systemic effect. The penetration depth of MN patches has been reported to be between one-half to two-thirds of the MN height (33). This is primarily due to the “bed of nails” phenomenon, where MNs require a high force per MN to penetrate the tissue due to the superelasticity of the skin (34, 35). To maximize drug delivery given these limitations, the MN preparation technique described in this manuscript localizes the payload in the MN tips. This, in turn, results in a more effective drug delivery method.

Our MN patches successfully delivered therapeutically relevant doses of HI to the buccal tissue of swine. HI-loaded MNs were stable when stored at 5° and 25°C for 3 months with low dimer formation. Trehalose did not have a significant effect as a stabilizer when added to the sorbitol formulation. The binding agent plays a key role in API stability; hence, each API may require formulation development as it will have to be paired with the most suitable formulation, and additional stabilizers could be added to help further stabilize the API (26). While hGH was detected in plasma from swine after PVP MN administration, a more compatible polymer will be needed as a binder to prevent hGH oxidation and deliver relevant therapeutic doses.

Prior studies have investigated the pain sensation associated with transdermal application of MN patches (36, 37). However, not all studies used an applicator to control the penetration force (38). We have designed an electronic-based applicator tailored to apply the patches to different tissues in the oral cavity in a reproducible manner. The clinical study revealed that the application of MNs on the palatal area causes less discomfort than on the arm. Volunteers chose the palate and the cheek as the preferred sites for patch application and indicated the sublingual space to be the most painful. It is important to note that we did not compare MN patch application to hypodermic needle placement. This potentially led to an overestimation of the pain scores reported for our patches. Nonetheless, 95% of participants would choose our technology to receive a flu shot, suggesting that MN arrays are preferred over conventional parenteral administration. Only 5% would opt for hypodermic needles primarily for their faster delivery capabilities. It should be noted that 60% of the participants in the study did not mind needles and yet preferred the MN patches (fig. S12). Therefore,
we anticipate that those who fear conventional needles would embrace the MN technology as means of delivery. In brief, the lower pain reported for palate application suggests that using MNs for buccal delivery could especially benefit pediatric populations, especially those constrained to daily injection therapies to treat certain medical conditions such as hGH deficiency or diabetes. The fast turnover of buccal mucosa could allow for daily use of the MN patches.

Successful translation of these MN systems will require further optimization from a manufacturing perspective to ensure that optimal formulations are identified for candidate APIs as evidenced by the differential degradation observed with certain API-polymer combinations. Previous studies confirmed the acceptability of MN patches by children (39), but further studies will be needed to evaluate the acceptability of buccal patches for drug administration compared with hypodermic needles in vulnerable populations such as pediatric patients.

**MATERIALS AND METHODS**

**Materials**

Stainless steel MN master structures containing 196 MNs (with a height of 1 mm and base diameters of 0.3 and 0.4 mm) were fabricated by Kjul & Co. Aps (Brondby, Denmark). Polydimethylsiloxane (PDMS) Sylgard 184 was purchased from Dow Corning (Midland, MI, USA). HI and hGH were obtained from Novo Nordisk (Måløv, Denmark). FITC-dextran with molecular weights of 3 to 5 kDa and 20 kDa, 97% trichloro(1H,1H,2H,2H-perfluorooctyl)silane, ≥98% D-sorbitol (food grade), L-histidine monohydrochloride monohydrate, and Sulforhodamine 101 acid chloride (Texas Red) were purchased from Sigma-Aldrich (St. Louis, MO, USA). PVP (M<sub>n</sub> 58,000 Da) was purchased from Alfa Aesar (Haverhill, MA, USA). Female Yorkshire swine were obtained from the Cummings School of Veterinary Medicine at Tufts University (North Grafton, MA, USA), and excised swine tissue was obtained from the Blood Farm Slaughterhouse (West Groton, MA, USA). Quantikine ELISA (enzyme-linked immunosorbent assay) Human Growth Hormone Immunoassay was purchased from R&D Systems Inc. (Minneapolis, MN, USA). Polycarbonate Makrolon 2458 was sourced from Covestro (Leverkusen, Germany), and injection was molded at Covestro (Leverkusen, Germany), and injection was molded at McMaster-Carr (Robbinsville, NJ, USA). Dental sleeves were sourced from the Amazon East Dental Company. Deionized water was used throughout the study.

**Fabrication of PDMS MN molds**

The stainless steel MN master structures containing 196 conical MNs were silanized under vacuum. Silicone molds were prepared by pouring a 11:1 mixture by weight of PDMS and binder onto the metal templates and placing them to cure at 65°C for 3 hours (Fig. 1C, i to iv).

**Fabrication of MNs with high drug loading**

To ensure a high loading amount of API in the MNs, the API in its solid form was dispersed into the PDMS mold and scraped into the MN cavities. Then, the molds were centrifuged at 4000 rpm for 10 min to force the API into the MN tips. A 50% (w/w) solution of PVP in deionized water or sorbitol melted at 100°C was poured into the mold as a binder and centrifuged at 4000 rpm for 10 min at 25°C. Molds were left to dry for 72 hours at room temperature, and then MN patches were demolded.

The same procedure was used to prepare MNs with FITC-dextran instead of API. In particular, FITC-dextran with an average molecular weight of 3 to 5 kDa was used to simulate HI, and a 22-kDa FITC-dextran modeled the size of hGH.

**Fabrication of MNs with Texas Red dye**

MN patches with Texas Red dye needle tips were prepared for MN insertion experiments. A 50% (w/w) PVP solution containing 0.02% (w/w) Texas Red dye was poured into MN molds and centrifuged at 4000 rpm for 10 min, and excess Texas Red solution that did not fill the MN tips was removed with a spatula. The solution that remained in the MN tips was left to dry for 24 hours. Molds were then filled with pure 50% (w/w) PVP solution, centrifuged at 4000 rpm for 5 min, and left to dry for 3 days before demolding. The resulting MN patches had Texas Red–PVP needle tips and a PVP baseplate.

**Imaging of MN arrays**

A Nikon A1R Ultra-Fast Spectral Scanning Confocal Microscope (Melville, NY, USA) was used to inspect the MN patches loaded with FITC-dextran. To image the MN, we used 20× air and 63× oil immersion objectives, and we detected the signal using a resonant scanner. Digital three-dimensional (3D) reconstructions were generated using a Nikon NIS Elements Acquisition Software based on approximately 1000 confocal slides per 3D image.

A JEOL 5600LV scanning electron microscope (Peabody, MA, USA) was used to image the MN, which had been previously coated with gold and palladium in a Hummer 6.2 Sputter Coating System (Anatech, Hayward, CA, USA). Images of the MN arrays for designated experiments were taken using a Leica M165 C stereo microscope with a coupled Leica DFC450 camera (Leica Microsystems, Buffalo Grove, IL, USA).

**Mechanical characterization of MNs and tissue penetration force**

Before starting the mechanical characterization tests, MN imaging was conducted on the stereo microscope as described in the previous section, and the camera images were processed using ImageJ (Open Source) to assess initial MN height. Mechanical testing of the penetration forces across various anatomical sites in swine and human tissues was performed using the Instron 5943 (Instron, Norwood, MA, USA) with a 500-N load cell. Swine tissue was harvested from freshly euthanized swine and tested within 1 hour postmortem. All tissue procurement protocols were reviewed by the Massachusetts Institute of Technology (MIT) Committee on Animal Care for animal tissue and the MIT Committee on the Use of Humans as Experimental Subjects for human tissue and deemed to be exempt. Human tissue from deceased organ donors was sourced from the National Disease Research Interchange (Philadelphia, PA, USA), who manage consent and processing of the tissue. The tissue was received on ice and tested within 48 hours of harvesting. Obtained human tissue samples were from the forearm. Buccal tissues were kept moist during testing to prevent dehydration. The tissue samples were placed on an acrylic and cork platform with a 1-cm-diameter through-hole in the middle to allow space for thin tissue to stretch. Thimbucks were used to keep small tissue samples from falling into the hole during testing. Hypodermic beveled needles of 30.5, 25, and 18 gauge (BD Franklin Lakes, NJ, USA) were advanced into the tissue at a rate of
0.1 mm/s. To account for individual variability, three trials (n = 3) of each needle gauge were conducted. For each and every penetration, brand new needles were used to ensure maximum needle sharpness and, therefore, consistency, and tissue was repositioned to prevent new needles from inserting into previously penetrated tissue locations.

**API quantification of the MN arrays**

For dissolution experiments, API-loaded MNs were scraped off from the baseplate using a scalpel and placed into a glass vial. HI-loaded MNs were dissolved in 1 ml of deionized water, and hGH-loaded MNs were dissolved in histidine solution (1.55 g/liter) before analysis. HI and hGH were then quantified using an Agilent 1260 Series high-performance liquid chromatography (HPLC) system (Santa Clara, CA, USA). HI separation was performed using a 7.8 mm–by–300 mm insulin HMWP column (Waters Corp, Milford, MA, USA) at room temperature. The mobile phase contained 15% acetic acid (v/v), 20% acetonitrile (v/v), and 1.8% phosphate buffer, and the API concentration and potential covalent dimer formation were quantified using the HPLC method previously described.

**In vivo MN-based drug delivery in swine**

Swine were prepared as described in the previous section. MN patches loaded with HI or hGH were inserted onto the palatal area or into buccal tissue of female Yorkshire swine with weights between 27 and 73 kg. Blood samples were withdrawn via the femoral vein at designated time points, including, but not limited to, every 5 min for the first hour, 10 min for the second hour, 15 min for the third hour, and every 30 min for the last hour. Blood glucose levels of swine dosed with HI-loaded patches were monitored at the time of blood withdrawal using a glucometer, and 10 ml of a dextrose 50% solution (VEDCO Inc., Saint Joseph, MO, USA) was administered via femoral catheter (bolus) or dextrose drip via central line in ear (diluted in 0.9% sodium chloride to 5%) if the swine’s glucose levels dropped below 20 mg/dl.

Drug delivery controls were prepared by dissolving API-loaded MN tips into a 0.9% sodium chloride saline solution (Hospira, Lake Forest, IL, USA), which was then filtered and injected subcutaneously to the swine. Blood samples were taken at designated time intervals over 2 hours.

Blood samples were collected into ethylenediaminetetraacetic K3 tubes (Sarstedt, Numbrecht, Germany), kept on ice until the last sample was collected, and then centrifuged at 4000 rpm for 10 min at 4°C. The supernatant (plasma) was then transferred to microtubes and shipped to Novo Nordisk (Maalov, Denmark) for analysis. HI was quantified using a Novo Nordisk in-house AlphaLISA assay optimized to specifically detect HI but not the endogenous swine insulin. hGH was quantified using Quantikine ELISA, a commercially available ELISA (R&D Systems Inc., Minneapolis, MN, USA).

**Stability studies**

Stability of the API-loaded MNs was tested under different conditions: (i) 5°C, (ii) 25°C, and (iii) 40°C, the latter at 75% RH. For this testing, MNs were packed separately into glass vials, which were, in turn, packed into DUMA containers (Gerresheimer, Dusseldorf, Germany) (fig. S13). Stability was tested immediately after preparation (time zero) and after 1, 2, and 3 months of storage. At these specified times, MNs were scraped off and dissolved in 4 ml of pH 3 phosphate buffer, and the API concentration and potential covalent dimer formation were quantified using the HPLC method previously described.

**Applicator design**

A special force-sensing applicator was designed with a handle to reach the different buccal spaces and ensure insertion of the patches at a predetermined and reproducible force of 10 N. The applicator (fig. S9) consists of a metal handle with a rubber pad (McMaster-Carr, Miami, FL, USA), and dextrose was administered when blood glucose levels dropped below 20 mg/dl. To examine the speed and extent of MN dissolution after insertion into the buccal tissue in vivo, we visualized HI-loaded MNs with the abovementioned stereo microscope before and after insertion. Furthermore, penetration of MNs into the tissue was confirmed via histology using hematoxylin and eosin stain of tangential sections.

To quantify the dye transfer in vivo, we inserted MNs with Texas Red needle tips into the swine’s buccal cavity for the same durations as previously described. Posteuthanasia, the buccal tissue was retrieved, and the fluorescence was visualized with an IVIS (in vivo imaging system) Spectrum (PerkinElmer, Waltham, MA, USA).
Preparation of sorbitol MNs and baseplates for the human study

Sorbitol was selected to fabricate the patches of this study given that it is a sugar widely used in regularly consumed products. Food-grade sorbitol was melted at 100°C and poured into PDMS molds to prepare patches with MNs and without MNs (baseplates). While the sorbitol was still molten, holders (described in the previous section) were affixed to each mold cavity, and the molten sorbitol would seep through specially designed overhangs, which serve to secure the sorbitol in place and prevent it from detaching from the holder. Upon cooling, the patches were demolded, transferred to six-well plates with fixtures to prevent movement, and then taped and put into a sterilization pouch with two sachets of desiccant. Each pouch contained two six-well plates, one with MNs and the other with baseplates. The patches were sterilized using gamma irradiation (Steris, Libertyville, IL, USA) with a Nordion Cobalt-60 Irradiator with a dose ranging between 25.76 and 27.89 kGy for an average of 347 min (40). Following gamma sterilization, sorbitol turned orange in color, and the irradiation of the holder resulted in a yellow tint, but this did not compromise the MN’s tip sharpness or the mechanical properties (fig. S8). On the contrary, gamma irradiation increased the mechanical strength, as reflected by a smaller MN tip compression observed when applying the same force (fig. S8A).

A limulus amebocyte lysate (LAL) Endosafe kit (Charles River, Charleston, SC, USA) was used to confirm the absence of endotoxins. Briefly, MNs and the first layers of the baseplates were dissolved in LAL reagent water in endotoxin-free glass tubes, and these test specimens were then mixed with equal parts of LAL reagent and incubated for 60 min at 37°C. The test specimens, together with a negative control (LAL reagent water) and a series of endotoxin controls based on four dilutions of control standard endotoxin, were run in duplicates as advised by the kit. After incubation, the tubes were inverted to assess the formation of a gel clot, which would indicate the presence of endotoxin.

Ex vivo and in vivo testing of the applicator

Before conducting the human trial, the penetration depth was tested ex vivo in swine and human tissue using OCT, an imaging technique that enables visualization of tissue penetration up to 2 mm in depth. A 1.3-μm-wavelength OCT system developed at MIT was used to visualize the MNs penetrated into tissue in time lapse. Furthermore, we applied MN patches in anesthetized swine using a force of 20 N to test for potential bleeding. Last, we applied baseplates to humans in the different locations defined in the next section to test the applicator and the force-sensing apparatus and the feasibility of applying the patches with a force of 10 N in a reproducible manner within less than 3 s.

Discomfort/pain assessment following MN application

The human study was approved by the MIT Committee on the Use of Humans as Experimental Subjects (COUHES) and performed under the auspices of MIT. Volunteers were recruited using posters displayed on the MIT campus (fig. S14) via distribution email lists and word of mouth. A total of 100 volunteers aged over 18 years were recruited for the study. Volunteers with any of the following conditions were excluded from the study: (i) mouth infections, including mouth sores, mouth ulcers, and gum inflammation; (ii) chronic or acute infections including HIV and hepatitis; (iii) immunodeficiency disease; (iv) diabetes; (v) condition that may cause excessive bleeding; (vi) currently taking any medication (example, coumadin), which causes excessive bleeding easily or susceptibility to infection; (vii) pregnant; or (viii) allergic to sorbitol. Eligibility was confirmed using REDCap (Research Electronic Data Capture) tools hosted at MIT (fig. S15). REDCap is a web-based software platform that supports data capture for research studies, providing (i) an interface for data capture, (ii) audit trails for tracking data manipulation and export procedures, (iii) automated data export procedures for statistical analysis, and (iv) procedures for data integration and interoperability with external sources (41).

Signed consent forms were obtained before the start of the study. Six sites of insertion were tested, namely, skin (forearm), tongue, sublingual, inner lower lip, cheek, and palatal area in four order patterns (table S1). Two patches were applied to each insertion site using the designed applicator: one with MNs and the other without. The pain assessment was conducted using a questionnaire based on the VAS score (28), which consists of a 10-cm line, where the left edge indicates “no pain,” and the right one “worst pain possible” (fig. S16). The volunteers were asked to draw a vertical line on top of the VAS score line to indicate their pain sensation. The VAS score was measured using a ruler and analyzed on Prism using the multiple t test. Statistical significance was determined using the Bonferroni-Dunn method, with α = 0.05. In addition, by filling a questionnaire, the participants expressed their preferred location of application in the mouth and their preference between our patches and hypodermic needles and wrote comments regarding the study/technology (optional open question).
Supplementary Materials

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/doi/10.1126/sciadv.abc3254.

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Competing interests: E.C.-S., D.M., N.R., R.L., and G.T. are coinventors on multiple patent applications describing oral biologic drug delivery. R.L. and G.T. report receiving consulting fees from Novo Nordisk. Complete details of all relationships for profit and not for profit for G.T. can be found at the following link: www.dropbox.com/sh/vmr4a2ajb56/AABsSNSIoq9MT1qLAE-T5a?dl=0. Complete details for R.L. can be found at the following link: www.dropbox.com/s/yc3qb5sl54v7x/Rev%20Langer%20COI.pdf?dl=0. The other authors declare that they have no competing interests. Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

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