Vaccination

An oral microjet vaccination system elicits antibody production in rabbits

Kiana Aran,1,2,3,4 Marc Chooljian,1,5,3 Jacobo Paredes,1,3,6 Mohammad Rafi,1 Kunwoo Lee,1,5 Allison Y. Kim,1 Jeanny An,1 Jennifer F. Yau, Helen Chum,7 Irina Conboy,1,5,4 Niren Murthy,1,5,3 Dorian Liepmann1,5,3*

Noninvasive immunization technologies have the potential to revolutionize global health by providing easy-to-administer vaccines at low cost, enabling mass immunizations during pandemics. Existing technologies such as transdermal micro-needles are costly, deliver drugs slowly, and cannot generate mucosal immunity, which is important for optimal immunity against pathogens. We present a needle-free microjet immunization device termed MucoJet, which is a three-dimensional microelectromechanical systems–based drug delivery technology. MucoJet is administered orally, placed adjacent to the buccal tissue within the oral cavity, and uses a self-contained gas-generating chemical reaction within its two-compartment plastic housing to produce a high-pressure liquid jet of vaccine. We show that the vaccine jet ejected from the MucoJet device is capable of penetrating the buccal mucosal layer in silico, in porcine buccal tissue ex vivo, and in rabbits in vivo. Rabbits treated with ovalbumin by MucoJet delivery have antibody titers of anti-ovalbumin immunoglobulins G and A in blood serum and buccal tissue, respectively, that are three orders of magnitude higher than rabbits receiving free ovalbumin delivered topically by a dropper in the buccal region. MucoJet has the potential to accelerate the development of noninvasive oral vaccines, given its ability to elicit antibody production that is detectable locally in the buccal tissue and systemically via the circulation.

INTRODUCTION

The potential of noninvasive vaccinations to provide low-cost mass immunization has been widely noted (1–4), but noninvasive vaccine delivery technology has been challenging to develop. Microneedles are currently the most promising platform for noninvasive vaccination but have many disadvantages: They require precise formulation for mechanical strength, as well as expensive fabrication and packaging. In addition, microneedles are inefficient at generating mucosal immunity, which functions as the first line of defense against infectious diseases that are initiated at mucosal surfaces and are consequently ineffective against a large number of infectious pathogens (4–7). Therefore, there is a great need for developing noninvasive mucosal vaccination strategies capable of inducing mucosal immunity together with high serum antibody titers for systemic immunity.

Vaccination via the oral route can effectively induce mucosal immune responses because of abundant presence of immune cells in the oral mucosa and is furthermore an ideal strategy for mass immunization due to its noninvasive nature, ease of administration, and high patient compliance. In particular, the buccal region of the oral cavity is very attractive for vaccination because of its distinct immunological features, such as high density of dendritic cells, Langerhans cells, T lymphocytes, and mucosa-associated lymphoid tissue, and is less prone to chemical and enzymatic degradation when compared to other oral vaccination targets such as the intestinal mucosa. (8–15). However, buccal vaccination has been challenging to implement because of the thick mucosal layer in the buccal cavity, which presents a substantial barrier to diffusion, and the limited permeability of the buccal epithelial cells to proteins (7–10, 12–20).

Here, we present a noninvasive vaccination technology, termed MucoJet, which generated high titers of antigen-specific immunoglobulins G and A (IgG and IgA), indicative of systemic and mucosal immune responses, respectively, after buccal administration in rabbits. MucoJet is a self-administered, two-compartment plastic device that generates a high-pressure liquid jet of vaccine with sufficient velocity to penetrate the mucosal layer of the buccal tissue, delivering the vaccine to underlying immune cells. This addresses the key problems that have prevented buccal vaccination from having clinical impact.

RESULTS

MucoJet is a two-compartment, 3D-printed plastic device

The first-generation MucoJet is a 15 mm × 7 mm cylindrical two-compartment plastic device (fig. S1A). The solid components were prototyped using a stereolithographic three-dimensional (3D) printer out of a photopolymerizable plastic resin, which is biocompatible and water-resistant (21, 22). The device is designed to be compatible with industry-scale thermoplastic fabrication methods such as injection molding, allowing for the use of a variety of inexpensive biocompatible materials.

The exterior compartment of the MucoJet is a chamber containing 250 μl of water (Fig. 1A). The interior compartment is composed of two reservoirs separated by a porous plastic membrane and a movable piston. The vaccine reservoir is a 100-μl chamber containing a vaccine solution with a piston at one end and a sealed 200-μm-diameter delivery nozzle at the other end (fig. S1A). The propellant reservoir, which contains a dry chemical propellant (citric acid and sodium bicarbonate), is separated from the vaccine reservoir at one end by the built-in porous membrane and movable piston and is sealed at the other end from the exterior compartment with a pH-responsive polymeric membrane with a dissolution threshold of pH 6.

The mechanism of MucoJet operation is shown in Fig. 1A. Immediately before administration in the buccal cavity, the interior and exterior components are injected of the photopolymerizable plastic resin, which is biocompatible and water-resistant (21, 22). The device is designed to be compatible with industry-scale thermoplastic fabrication methods such as injection molding, allowing for the use of a variety of inexpensive biocompatible materials.

1Department of Bioengineering, University of California (UC), Berkeley, Berkeley, CA 94720, USA. 2School of Applied Life Sciences, Keck Graduate Institute, Claremont, CA 91711, USA. 3Berkeley Sensor and Actuator Center, Berkeley, CA 94720, USA. 4Buck Institute for Research on Aging, Novato, CA 94945, USA. 5UC Berkeley–UC San Francisco Graduate Program in Bioengineering, Berkeley and San Francisco, CA, USA. 6CEIT and Tecnun (University of Navarra), 20018 San Sebastián, Spain. 7Office of Laboratory Animal Care, UC Berkeley, Berkeley, CA 94720, USA.

*Corresponding author. Email: karan@kgi.edu (K.A.); liepmann@berkeley.edu (D.L.); nmurthy@berkeley.edu (NJM)
compartments of the MucoJet are clicked together. This results in dissolution of the polymeric valve membrane sealing the propellant reservoir. Water contacts the chemical propellant in the propellant reservoir, triggering a chemical reaction that generates carbon dioxide (CO₂) gas. The gas production increases the pressure in the propellant chamber, causing the piston to move. The free-moving piston ensures uniform movement of ejected drug and blocks the exit of effervescence generated by the CO₂ through the nozzle (fig. S2). When the pressure in the propellant chamber reaches ~30 kPa, the force on the piston is sufficient to break the nozzle seal of the vaccine reservoir. The vaccine solution is then ejected from the MucoJet nozzle, penetrates the mucosal layer of the buccal tissue, and delivers the vaccine to underlying APCs (Fig. 1B).

**Jet velocity and pressure are sufficient to penetrate the buccal mucosal layer in simulations**

MucoJet was designed to deliver vaccines to the buccal immune system, and therefore, the generated jet of vaccine must have sufficient momentum to penetrate the mucosal layer. To measure the pressure of the vaccine jet output by the MucoJet device, we inserted a pressure sensor adjacent to the coated nozzle within the vaccine reservoir (fig. S3). Pressure within the vaccine reservoir was monitored in real time and showed that the average pressure generated in the chamber increased to 25 to 30 kPa before the nozzle broke, indicated by a sharp drop in recorded pressure. Time-lapse image analysis was used to calculate ejection velocity, with the output jet estimated at 50 to 200 mm/s for different prototypes (movie S1 and fig. S4).

We performed numerical simulations of the device to acquire preliminary evidence that the pressure generated by the chemical propellant was sufficient to drive fluid through the mucosal layer. A three-phase fluid analysis was used to model the transport of the vaccine across the lumen and the mucus of the buccal space, and the Navier-Stokes equations were solved using constant pressure boundary conditions at the inlets and outlets (see Simulation methods and fig. S5 for detail of the simulation domain, showing the initial distribution of the mucus and lumen phases). The third phase, vaccine-carrying fluid, is initially zero everywhere in the domain and is injected through the pressure inlet representing the device nozzle. Mucus was modeled as a power-law shear-thinning fluid using parameters taken from the literature, and the thickness of the mucosal layer was set to 800 μm (23). The simulations conducted using pressure boundary conditions corresponding to the measured pressure output of the MucoJet device (30 kPa; fig. S3) suggested that MucoJet was capable of penetrating the buccal mucosal layer (Fig. 2A). Furthermore, simulations using 10 kPa—one third of the measured pressure output—still showed rapid penetration of the mucosal layer, suggesting that there was considerable opportunity for engineering optimization.

In addition, simulations showed penetration of the buccal mucosal layer within 0.4 ms as shown in Fig. 2B, where the spike in pressure indicates impact of the jet with the endothelial surface. In contrast, hydrophilic small molecules have a characteristic diffusion time of at least 10 min across an 800-μm mucosal layer, and this diffusion time is significantly higher for macromolecules of the size of antigens (24). Therefore, the demonstrated ability of the jet to penetrate the mucosal layer via convection could enhance the rate of fluid transport across the buccal mucosa by several orders of magnitude through bypassing the diffusion barrier. The rapid rate of transmucosal connection also minimizes diffusive losses of vaccine into the buccal interior. The Péclet number calculated on the basis of the simulation is about 4 × 10⁷ (see Simulation methods), demonstrating that convective transfer of the vaccine through the mucosal layer happens much faster than diffusive transport out of the jet. Furthermore, the simulated pressure at the epithelial cell layer was roughly equal to the nozzle pressure (Fig. 2B), suggesting that
Fig. 2. Computational fluid dynamics modeling of MucoJet-generated output pressure and buccal mucosal penetration. (A) ANSYS Fluent simulation showing simulated vaccine jet with nozzle pressures of 10 and 30 kPa penetrating the simulated mucus. Heatmaps of drug volume fraction from zero (no drug) to one (drug concentration is the same as the reservoir concentration) use a linear color scale (zero, blue; one, red). The arrow indicates the location of the pressure measurements shown in (B). Bottom left: Backflow can be seen in (A) as red spirals of vaccine-carrying fluid at the edge of the pill body. (B) Plots of pressure at the epithelium surface (left) and jet velocity (right) versus time for nozzle pressures of 30 kPa (green) and 10 kPa (blue). Pressure briefly spikes upon impact of jet with buccal wall and then levels off at nozzle pressure, showing negligible pressure loss. (C) Plots of volume delivered within the mucosal layer (left) and efficiency of delivery to the mucosal layer (right) over time. Efficiency is calculated as the fraction of total drug volume delivered that is within the mucosal layer.

Fig. 3. MucoJet-delivered protein solution penetration of porcine buccal tissue in vitro. (A) Illustration depicting the experimental setup for the ex vivo Transwell assays. Fresh porcine buccal tissue was placed atop the membrane insert (apical side). Ovalbumin was delivered via MucoJet or dropper to the apical compartment and medium solution sampled every 30 min from the basolateral compartment. Ovalbumin solution is shown in blue, and ovalbumin proteins are shown in blue dots. (B) Percentage of ovalbumin delivered by MucoJet or dropper (control) across porcine buccal epithelium over time as measured by fluorescence spectroscopy. Data are means ± SEM (n = 3). (C) Western blot analysis of ovalbumin in the buccal tissue after delivery by MucoJet at two different ejection pressures [30 to 40 kPa (n = 4) and 10 to 15 kPa (n = 4)] and via a dropper (n = 3). Data are means ± SEM (n = 4).
the local pressure at the epithelial cell wall would be elevated to the pressure of the vaccine reservoir during delivery. Because a link between elevated hydrostatic pressure and increased epithelial permeability has been documented in the literature, this could enhance epithelial uptake of the vaccine (25).

Delivery efficiency was quantified as a function of time by comparing the amount of drug-carrying fluid within the mucosal layer to the total amount delivered by the device (see Fig. 2C). The simulations showed an initially high delivery efficiency, but the efficiency decreased as the area beneath the device filled with drug-carrying fluid and fluid began backflowing into the buccal space. The amount and timing of backflow and therefore the delivery efficiency are functions of the size of the device and the delivery pressure.

**Mucojet enhances the delivery of ovalbumin to porcine buccal tissue in vitro**

To determine whether protein solutions ejected from Mucojet had sufficient velocity to penetrate the buccal tissue, we performed ex vivo experiments to measure delivery of protein through pig buccal tissue samples. As shown in Fig. 3A, freshly prepared porcine buccal epithelium was mounted in Transwell chambers, and a solution of fluorescein-labeled ovalbumin (300 μg of ovalbumin–fluorescein conjugate in 100 μl of water) was delivered to the top of the buccal mucosa (apical chamber) using either Mucojet (n = 3) or a dropper (n = 3). The amount of ovalbumin that passed through the buccal tissue was measured in the basolateral chamber. Figure 3B demonstrates that Mucojet significantly (P = 0.0004) enhanced the delivery of ovalbumin across the buccal tissue, causing an eightfold increase in the delivery of ovalbumin over the course of 3 hours compared to topical dropwise application. Similar studies were performed to evaluate the effect of pressure on the amount of ovalbumin delivered into the tissue. Immediately after ovalbumin delivery, the amount of ovalbumin within the buccal tissue was quantified using Western blotting. The results indicated that increasing the Mucojet output pressure from 0 to ~15 kPa (n = 4) significantly (P = 0.033) enhanced ovalbumin delivery into the tissue. Increasing the pressure to ~30 kPa (n = 4) further increased the ovalbumin delivery to the tissue as compared to 15 kPa, indicating that the delivery efficiency is a function of delivery pressure (Fig. 3C).

**Mucojet increases ovalbumin delivery to rabbit buccal mucosa in vivo and enhances both systemic and mucosal immunogenicity**

Having shown that Mucojet enhanced protein delivery to the buccal tissue in vitro, we next sought to determine whether Mucojet could enhance the transport of vaccine and immunogenicity of buccally delivered antigens in vivo. Ovalbumin was chosen as a model vaccine because it is a standard nontoxic reference protein for studying antigen-specific immune responses in animals (26–28). New Zealand white (NZW) rabbits were vaccinated with Mucojets containing 100 μl of ovalbumin solution for a dose of 100 μg/kg at week 0 and were boosted with the same dose at week 4 (n = 4). Control groups received the same dosages of ovalbumin using a dropper delivery method at the buccal site (n = 4) at the same time points (weeks 0 and 4). For the Mucojet-treated group, the device was removed from the oral cavity immediately after deployment of the vaccine payload (within ~10 s).

Blood samples were collected weekly for 6 weeks from the rabbits’ marginal ear veins, and blood antibody titers for ovalbumin-specific serum IgG were quantified. After 6 weeks, rabbits were euthanized and the buccal tissue, Peyer’s patches, and mandibular lymph nodes were harvested to determine ovalbumin-specific IgA expression. Mucojet enhanced the immunogenicity of buccally delivered antigens as assessed by both blood- and tissue-specific titers (Fig. 4, A and B). Rabbits treated with ovalbumin by Mucojet delivery had antibody titers of IgG and IgA that were three orders of magnitude higher than titers from rabbits treated by buccal administration of free ovalbumin. In addition, the second introduction of ovalbumin by the Mucojet (week 5) resulted in a significant (P = 0.038) increase (about sevenfold) in anti-ovalbumin IgG production as compared to the initial ovalbumin-specific IgG response to the Mucojet (week 1) (Fig. 4A). Such an Ig “boost” is indicative of the induction of adaptive immunity (29).

To better understand the mechanism of protein delivery, we delivered 100 μl of ovalbumin (100 μg/kg) to rabbits using Mucojet or topical dropper application as described above. Animals were euthanized within 2 to 3 hours after ovalbumin administration, and buccal tissue samples were harvested. The amount of ovalbumin in buccal tissue samples was quantified using Western blot. Mucojet delivery resulted in a sevenfold increase in the penetration of buccally delivered ovalbumin in vivo.
can be used to enhance the transport of macromolecules. In addition, the histopathological evaluation of the buccal tissue after delivery is normalized to positive control. Data are means ± SEM (n = 3).

Fig. 5A). Figure 5B shows representative images of the rabbit tissue damage in either the MucoJet- or dropper (control)–treated rabbits. Tissue was isolated 2 to 3 hours (C) and 6 weeks (D) after administration. Difference in image quality is due to different thicknesses of the samples. Scale bar, 200 μm.

DISCUSSION

MucoJet is a buccal drug delivery system 3D-printed from biocompatible plastic that can enhance the systemic and mucosal immunogenicity of protein antigens because of its ability to generate a vaccine-carrying jet that can penetrate the buccal mucosal layer. Increased ovalbumin transport due to MucoJet delivery was similar to transport enhancements that can penetrate the buccal mucosal layer. Increased ovalbumin translocation due to MucoJet or dropper (control)–treated rabbits. Tissue was isolated 2 to 3 hours (C) and 6 weeks (D) after administration. Difference in image quality is due to different thicknesses of the samples. Scale bar, 200 μm.

Fig. 5. Ovalbumin penetration into the buccal tissue in vivo. (A) Western blot analysis of ovalbumin in the buccal tissue after delivery by MucoJet or dropper. Percentage of ovalbumin delivery is normalized to positive control. Data are means ± SEM (n = 3). (B) Images of the buccal delivery site in rabbits (n = 3) 2 to 3 hours after vaccine delivery with MucoJet (left) or dropper (control) (right). (C and D) Histopathological evaluation of the buccal tissue at the site of ovalbumin delivery in MucoJet- and dropper (control)–treated rabbits. Tissue was isolated 2 to 3 hours (C) and 6 weeks (D) after administration. Difference in image quality is due to different thicknesses of the samples. Scale bar, 200 μm.

There are several limitations in our study that invite future work. First, effective vaccine dosage and immune responses (antibody titers) required for mucosal and systemic protection can differ vastly, depending on the individual pathogen (31). Therefore, in-depth studies on protective immunity using multiple vaccines administered by MucoJet delivery, performing immune challenge experiments to better understand the dosing required to achieve protective immunity against different diseases, should be conducted. Second, to better understand the fluid dynamics made in the simulation, turbulent dissipation due to the larger length scale in the velocity measurement experiment compared to both the simulation and the normal operation of the device (pressed against the buccal surface), and the method of jet measurement, which measured the propagation of the end of the jet rather than the instantaneous fluid velocity at the center of the jet. A more accurate determination of the velocity distribution (and thus the peak velocity at the center of the jet) could be made using digital particle image velocimetry experiments, but this is unnecessary, given the support of the in vitro and in vivo biological experiments establishing the efficacy of the device, and is therefore outside of the scope of this study. We believe that the simplifying assumptions we made to conduct our simulations are reasonable in light of the incredibly short time scale of the jet, reaching the buccal wall within several milliseconds, and the comparatively small size of the tissue microstructure when compared to the mucosal layer. Although the pressure at the nozzle decays as the piston moves, expanding the volume of the propellant gas, we assumed that nozzle pressure was constant. Because the time scale over which the mucosal layer is penetrated (several milliseconds) is very small compared to the time scale over which the pressure changes (hundreds of milliseconds) and the pressure is very close to constant over the time domain of the simulation, our assumption of constant pressure at the nozzle is valid.

Engineering optimization of the delivery pressure and the device geometry could further enhance the transport of proteins into the buccal tissue and perhaps into the systemic circulation, making MucoJet a useful candidate for oral-to-systemic delivery of biologics. However, increasing the pressure beyond a certain point could also result in increased backflow of the drug and decreased delivery rate. The simulations suggest a relationship between the pressure, the rate of drug delivery, and the delivery efficiency. An increase in pressure will increase the flow rate of the jet and therefore deliver drug-carrying fluid into the mucosal layer more quickly. Because the space between the nozzle and the buccal surface is filled, additional flow will push drug-carrying fluid out of the mucosal layer and back into the buccal cavity. Because our simulations also suggested that MucoJet could be effective at much lower pressures than currently used on the basis of the rapid penetration of the mucosal layer even at 10 kPa, there is considerable space for optimization.

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involved in MucoJet delivery and to develop engineering improvements on the device design, more complex simulations could be conducted. Areas in which the simulations could be improved include the use of more complex viscoelastic material models for the mucosal layer and simulation of a longer time scale, which would likely require modeling of diffusion and pressure loss. Although our simulations were well supported by in vitro and in vivo experiments, improved simulations could provide further insight into optimization of MucoJet and further understanding of the behavior of jets in multiphase non-Newtonian fluid environments.

We have shown that MucoJet can significantly enhance the immunogenicity of ovalbumin, a standard vaccine model, by monitoring ovalbumin-specific IgA and IgG responses in rabbits. In doing so, we have shown that MucoJet is capable of eliciting both systemic and mucosal responses against ovalbumin without the use of toxic adjuvants, which suggests that high pressure can enhance vaccine penetration into the mucus, and therefore has tremendous potential as a platform for mass immunization given its high efficacy and safety. MucoJet is superior to minimally invasive microneedle technology in that it induces mucosal immunity and therefore has the potential to be more effective in targeting pathogens whose route of entry is the mucosa. In addition, MucoJet can be designed to store the vaccine in a lyophilized form where the vaccine is reconstituted with a diluent before administration to increase the shelf stability. The MucoJet components themselves are simple and compact and can be fabricated from robust plastic materials, allowing for easy storage, transportation, and assembly. Furthermore, MucoJet can be designed in various shapes, sizes, and designs to simplify vaccine administration procedures and increase patient compliance, especially for children (for example, MucoJet in a shape of a lollipop). Finally, these studies also represent an effective proof of concept for this drug delivery technology and suggest directions for future improvement for oral delivery of variety of biologics.

**MATERIALS AND METHODS**

**Study design**

The objective of this work was to engineer an oral noninvasive vaccination system, MucoJet, to overcome the limitations associated with oral delivery of vaccines and improve vaccine immunogenicity without the use of toxic adjuvants. MucoJet was designed to generate a high-pressure liquid jet of vaccine with sufficient velocity to penetrate the mucosal layer of the buccal tissue and was fabricated using a biocompatible material and a 3D printer. Experiments were performed to monitor the jet pressure and the velocity profile of the device. Simulation studies were performed to verify that the measured pressure was sufficient to penetrate the mucosal layer. Ex vivo experiments on pig buccal tissues (n = 3) were designed to validate the results of the simulations, thereby determining whether the generated output pressure could enhance the penetration of ovalbumin into the buccal tissue. In vivo randomized experiments using rabbits (n = 4) were performed to evaluate whether MucoJet could enhance the immunogenicity of ovalbumin and elicit mucosal and systemic immune responses. Primary data are reported in table S1.

**Design and fabrication of MucoJet devices**

MucoJet is a 3D-printed plastic device (15 mm × 7 mm) designed with computer-aided design software (Creo Parametric, PTC; see auxiliary). Our prototype devices were 3D-printed in plastic-like material by a stereolithographic EnvisionTEC printer. The printing material, E-Shell 300 3D, was supplied by EnvisionTEC. This material is certified for medical applications and is biocompatible and not biodegradable (27–29). Ghostly S1A shows a schematic of the MucoJet. The interior body consists of two compartments, the propellant and the vaccine reservoirs, separated by a porous built-in membrane. A lid is attached to the main body of the device to hold the sterile vaccine within the vaccine reservoir. A mixture of sodium bicarbonate and citric acid (Sigma-Aldrich) is used as gas-generating chemicals stored as propellant powders in the propellant reservoir. The vaccine reservoir can hold about 100 μL of lyophilized vaccine solution. Alternatively, the vaccine can be stored in its lyophilized powder form inside the vaccine reservoir and solubilized immediately before administration to enhance the stability of the vaccine.

A schematic of the assembly process is shown in fig. S1B. The propellant reservoir is sealed with a Eudragit S-100 (Evonik Industries)–coated gelatin shell at one end and the separating membrane and the piston at the other end. The free-moving piston is placed atop the separating membrane inside the vaccine reservoir to ensure uniform movement of ejected drug and to block the exit of effervescence through the nozzle (fig. S2). The nozzle is coated with a thin layer of lacquer, which holds the vaccine inside the vaccine reservoir. The lacquer layer is designed to break at pressures ranging 25 to 30 kPa.

The final MucoJet assembly, where the interior body of the device is placed inside the exterior water-containing compartment, is simple and is intended to be performed immediately before administration. MucoJet is versatile and can be designed to hold various vaccine dosages within various device geometries. In addition, the delivery pressure can be modified by changing the amount of propellant, the thickness of the nozzle coating, and the diameter or number of nozzles. The activation time of the MucoJet can also be modified by optimizing the thickness of the pH-responsive membrane coating (Eudragit L100-55) sealing the propellant reservoir.

**Simulation methods**

To establish whether the measured pressure was sufficient to drive flow through the mucosal layer and to establish design rules governing the pressure and velocity of the microjet flow, we conducted computational fluid dynamics simulations. Simulations were conducted in ANSYS Fluent 15.0 using a 2D axisymmetric model with a transient (time step, 10−3 s) pressure-based solver. An implicit volume of fluid model was used for multiphase modeling, and laminar flow was assumed. Three phases were modeled: lumen, mucus, and drug-carrying water (fig. S5). Diffusion of drug was ignored, which is justified because of the very high Péclet number of the system (~4 × 107; using Pe = UL/D, with L = 800 μm, U = 5 m/s, and D on the order of 10−6 cm/s). A Péclet number of this magnitude indicates that convection is the dominant transport process in the system and that the effect of diffusion is negligible in the time scales simulated (32).

To generalize the model to a variety of delivered drugs, no drug concentration was used in the model, which is possible because of the lack of diffusive transport. Instead, the drug-carrying fluid was assumed to carry a concentration of drug equal to the concentration in the MucoJet reservoir, and the concentration was assumed to be zero everywhere else. This assumption holds across a large range of concentrations, as long as the concentration is not so large as to change the properties of the fluid. An implicit volume-of-fluid model was used with drug-carrying fluid modeled as a distinct phase with identical mechanical properties to water, and the volume fraction of this phase was plotted spatially (fig. 2). The concentration of drug can be calculated from this...
data by simply multiplying the drug concentration in the MucoJet by the volume fraction, which varies from 0% everywhere in the domain at initialization to 100% at the nozzle (fig. S4A).

Mucus was modeled as a non-Newtonian power-law fluid according to the equation

$$\eta = k \gamma^n$$  \hspace{1cm} (1)

where the consistency index $k = 1.412 \text{ kg s}^{-2/3}$, the power-law index $n = 0.15$, $\gamma$ is the shear rate, and $\eta$ is the viscosity. A maximum viscosity limit of 10 kg/m s was used to prevent solution divergence in areas with zero shear rate. These parameters were taken from literature (19). A pressure boundary condition 125 $\mu$m away from the mucosal surface, simulating close but not in perfect contact with the surface of the buccal mucosa, and 250 $\mu$m in diameter was used to simulate the ejection of drug from the MucoJet. A number of simplifying assumptions were made in designing this model. The walls of the buccal surface were treated as rigid, immobile, and flat, ignoring tissue motion, the deformability of the buccal tissue, and the tissue microstructure. Bulk flow in the mouth was also ignored, and the pressure at the nozzle was assumed to be constant.

**Ex vivo investigation of protein delivery using porcine buccal mucosa**

We used a standardized Transwell (Sigma-Aldrich) assay model system to quantify the kinetics of drug release from MucoJet by monitoring the permeation into and through pig buccal mucosal tissue in vitro. Briefly, freshly excised porcine buccal tissue ($n = 3$) was harvested from adult domestic medium-sized pig heads obtained from a local abattoir (Marin Sun Farms Inc.) immediately after slaughter. The tissue was transferred to the laboratory in ice-cold Krebs-Ringer bicarbonate solution (pH 7.4; Sigma-Aldrich). To prepare tissue specimens, we removed the underlying connective tissue and fat with a scalpel blade and surgical scissors within 2 hours postmortem. The buccal mucosa was sliced using a tissue slicer (Fisher Scientific) to obtain consistent tissue thickness of 0.5 mm. Skin biopsy punch devices (Fisher Scientific) were used to cut freshly excised tissue slices into circular specimens (diameter, 7 mm; thickness, 0.5 mm). The thickness of the tissue slices was measured using a caliper digital micrometer (Fisher Scientific) to verify the target thickness and minimize variations among the samples. A Transwell assay was constructed by placing the buccal tissue specimen onto a Transwell polycarbonate membrane cell inserts (Sigma-Aldrich) and adding phosphate-buffered saline (PBS) (Sigma-Aldrich) to the apical and basolateral compartments as shown in Fig. 3A. Ovalbumin (300 $\mu$g of ovalbumin-fluorescein conjugate in 100 $\mu$l of water) was delivered to the buccal tissue in the apical compartment via MucoJet or dropper (control) (1-ml plastic dropper; Fisher Scientific), and samples were taken every 30 min from the basolateral compartment. The fluorescence absorbance of medium samples was analyzed using a microplate reader to quantify the amount of ovalbumin that diffused across the tissue.

Similar Transwell experiments were performed to monitor the amount of ovalbumin that diffused into the tissue immediately after ovalbumin administration. To monitor the effect of pressure on the ovalbumin delivery rate, we generated two different pressures [~10 to 15 kPa ($n = 4$) and ~30 to 40 kPa ($n = 4$)] in the MucoJet by modifying the thickness of the nozzle coating using single- and triple-layer lacquer coatings, respectively. Five minutes after ovalbumin delivery via MucoJet or dropper (control; $n = 3$), the buccal tissue samples were removed from the Transwell chamber, washed three times for 5 min each with PBS (Sigma-Aldrich), and analyzed by Western blotting.

**Quantification of protein using Western blot**

Porcine buccal tissue samples were cut into small pieces, transfected into a vial containing 2 ml of lysis buffer (1:100 radioimmunoprecipitation assay buffer in phenylmethylsulfonyl fluoride; Sigma-Aldrich) on ice, and homogenized using a tissue homogenizer. After 2 hours of incubation at 4°C on an orbital shaker, the resulting suspension was centrifuged at 12,000 rpm for 20 min while maintaining a temperature of 4°C, and the supernatant was aspirated, discarding insoluble materials. Total protein concentration for each sample was determined (NanoDrop 2000, Thermo Scientific), and 20 $\mu$g of protein was transferred to a separate tube with loading buffer and heated for 10 min at 100°C. Tissue protein extracts (20 $\mu$g per lane) were run on 4 to 20% SDS-polyacrylamide gels and transferred onto polyvinylidene difluoride membranes (Mini-PROTEAN TGX, Bio-Rad). Blots were subsequently blocked in 3% bovine serum albumin in PBS solution and were treated with primary antibodies overnight at 4°C (anti-ovalbumin, 1:1000; Abcam), followed by treatment with horseradish peroxidase (HRP)--conjugated secondary antibody for 90 min at room temperature. Protein bands were visualized with Pierce Enhanced Chemiluminescence Western Blotting substrate (Pierce Biotechnology Inc.). Quantification of protein bands was performed with ImageJ software, and the results were normalized to a positive control consisting of 300 $\mu$g of ovalbumin added to a sample from an untreated tissue.

**Ovalbumin delivery to rabbits in vivo and measurement of immune response**

NZW rabbits weighing about 2.5 to 3.5 kg were used in accordance with protocols approved by the University of California, Berkeley Animal Care and Use Committee. After sedation of the rabbits by subcutaneous administration of acepromazine (1 mg/kg) (Sigma-Aldrich), ovalbumin (EndoFit, InvivoGen) was delivered to buccal mucosal surface (100 $\mu$g/kg in 100 $\mu$l of sterile water) by assembling the MucoJet as described above and placing a MucoJet device into the oral cavity against the rabbit’s cheek (week 0). MucoJet was removed from the oral cavity immediately after activation and vaccine delivery within 10 s after inserting into the oral cavity ($n = 4$). Rabbits receiving the same amount of ovalbumin via a dropper placed close to the surface of buccal tissue served as controls ($n = 4$). At week 4, a booster dose of ovalbumin (100 $\mu$g/kg) in 100 $\mu$l of sterile water was given to both experimental and control groups using the same delivery methods as the initial exposure.

Before each experiment, 200 $\mu$l of blood was collected from the marginal ear vein to quantify each animal’s starting blood for ovalbumin-specific IgG concentration. After the initial collection, blood samples were collected weekly for 6 weeks. Plasma was separated by centrifugation (3500 rpm; 15 min, 4°C) and was analyzed using ELISA for ovalbumin-specific IgG antibodies. Animals were euthanized by intravenous injection of pentobarbital (390 mg/10 pounds; Sigma-Aldrich) at week 6 and, after euthanasia, tissue collection of the lymph nodes, Peyer’s patches, and buccal tissue from the experimental and control rabbits were performed with assistance from a veterinary personnel. Tissues were rinsed with 1× PBS to remove excess blood, cut into 100-mg pieces, homogenized, and stored overnight at ~20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 10 min at 7000g. The supernatant was then removed and stored at ~80°C until needed for ELISA analysis.

ELISAs were performed to determine anti-ovalbumin IgG antibodies in rabbit serum and anti-ovalbumin IgA antibodies in tissue. To prepare the ELISA plate for analysis, we coated ovalbumin on a Nunc MaxiSorp flat-bottom 96-well plate (Thermo Scientific) by adding
50 μl of ovalbumin (5 μg/ml) into each well. After incubating the plate overnight at 4°C, the ovalbumin was gently removed from the wells using a pipette while tilting the plate as to not disturb the bottom of the wells. The plate was then washed two times by adding 200 μl of PBS containing 0.5% Tween 20 (Sigma-Aldrich) to each well, flipping over the plate, and gently tapping it on a paper towel to remove all the solution. Next, 200 μl of 5% dry milk in PBS solution was added to each well to prevent nonspecific adsorption on the well plate, and the plate was incubated for 2 hours at room temperature. After incubation, the solution was removed from the wells using a pipette, and the plate was washed two times with 200 μl of PBS Tween 20 (PBST), as described above. The rabbit serum samples and tissue samples were then diluted to concentrations of 1:10, 1:100, 1:1000, and 1:10,000 with PBS, and 100 μl of each diluted sample was added to the plate wells (three wells per sample). The plate was then incubated for 2 hours at room temperature and washed two times with PBST. Next, 100 μl of HRP-conjugated bovine anti-rabbit IgG (Santa Cruz Biotechnology) or HRP-conjugated goat anti-rabbit IgA (Thermo Scientific) was added into the plate to detect IgG and IgA antibodies in serum and tissue, respectively, and the plate was incubated for 30 min at room temperature. The plate was then washed, and 100 μl of fluorescent-labeling agent (AM1, Active Motif) was added to each well, allowing the color to visibly develop to the naked eye. Finally, 100 μl of stop solution (TransAM, Active Motif) was added to each well to stop the reaction. The plate was read using a plate reader at a wavelength of 450 nm.

In addition, experiments were performed to monitor transport of ovalbumin into the buccal tissue. Briefly, ovalbumin was delivered to the rabbits using MucoJet (n = 3) or dropper (n = 3) administration as described above. The rabbits were euthanized 2 to 3 hours after ovalbumin administration, and the buccal tissue (~1 cm × 1 cm × 5 mm) was harvested and washed with PBS. The tissue samples were analyzed using Western blotting.

Histology
To assess the safety of MucoJet delivery, we monitored the integrity of the buccal mucosa for acute and prolonged tissue damage in MucoJet- and dropper (control)–treated groups. MucoJet- and control–treated rabbits were sacrificed 2 to 3 hours after vaccine delivery to evaluate acute effects and 6 weeks after delivery to evaluate prolonged effects. After sacrifice, the oral cavity and the buccal area were visually examined for evidence of bleeding. Buccal tissue samples were collected, washed three times with PBS, fixed with 10% buffered formalin solution, immersed for 12 hours in 30% sucrose (Sigma-Aldrich) solution until the sample dropped to the bottom of the solution, and then embedded in Tissue-Tek optimum cutting temperature solution. Histological examination was performed using a cryomicrotome (HistostatCryostat Microtome). Buccal sections on glass slides were stained with hematoxylin and eosin (Molecular Machines & Industries) according to the manufacturer’s instructions, and the images (~10 images per sample) were examined using a microscope (Zeiss Axio Observer epifluorescence microscope).

Statistical analysis
Statistical analysis was conducted in Microsoft Excel. Because our previous hypothesis predicted an increase in antibody titer and ovalbumin concentration, the use of a one-tailed t test is appropriate, and accordingly, a one-tailed t test assuming unequal variances was used. P values listed above bars indicate a comparison of experiment versus control for that time point. Error bars on all plots represent means ± SEM. P ≤ 0.05 was considered statistically significant.

SUPPLEMENTAL MATERIALS
www.sciencetranslationalmedicine.org/cgi/content/full/9/380/eaaf6413/DC1

Materials and Methods
Fig. S1. Full schematics of the MucoJet and its assembly.
Fig. S2. Mechanism of MucoJet nozzle sealing after delivery of the vaccine to prevent the release of effervescence.
Fig. S3. MucoJet prototype and experimental setup to measure the pressure generated in the MucoJet.
Fig. S4. MucoJet ejection and estimation of average jet velocity.
Fig. S5. Detail of simulation domain.
Table S1. Primary data.
Movie S1. A video of liquid jet generated by MucoJet.
MucoJet CAD files

REFERENCES AND NOTES
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Abstracts

One-sentence summary: A needle-free device delivers a liquid jet of vaccine that penetrates the buccal mucosa and elicits antibody production in rabbits.

Editor’s Summary: Time for a booster shot? Open wide instead

No one likes to be on the receiving end of a needle, which can make routine childhood vaccinations especially problematic. Aran et al. developed a needle-free drug delivery device that can be administered orally. The MucoJet device used a simple chemical reaction to deliver a jet of vaccine—in this case, ovalbumin—that penetrated the buccal mucosa when placed against the cheek inside the oral cavity in rabbits. The rabbits showed evidence of anti-ovalbumin antibodies in cheek tissue and ear vein blood draws up to 6 weeks after vaccination.