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Supplementary information

Cephalopod-inspired jetting devices for gastrointestinal drug delivery

In the format provided by the authors and unedited

Methods

1. Theoretical mechanics of jetting

We modeled the ideal jetting system shown in Extended data fig. 1a with constant ampule pressure, p_{in}, piston stroke, L, piston diameter, d_p, piston velocity, v_p, jet area diameter, d_j, and jet velocity, v_j. According to the Bernoulli energy balance (assuming loss, gravitation and input kinetic energy terms are neglected) the velocity of a jet can be determined as follows:

$$p_{in} = \frac{1}{2}\rho {v_j}^2 \;\rightarrow\; v_j = \sqrt{\frac{2 \cdot p_{in}}{\rho}} \tag{E1}$$

If we assume conservation of volumetric flow and that the system is operating at steady state, it follows that the theoretical period of jetting, T, is as follows:

$$T = L \cdot \frac{A_p}{A_j}\sqrt{\frac{\rho}{2 \cdot p_{in}}} \tag{E2}$$

The theoretical thrust of the jet, F_j, is equal to the mass flow, $\dot{m}$, multiplied by the jet velocity, as follows:

$$F_j = \dot{m} \cdot v_j = \rho \cdot A_j \cdot {v_j}^2 = 2 \cdot A_j \cdot p_{in} \tag{E3}$$

where the jet velocity is determined from Equation E1. Finally, the theoretical power of the jet, P_j, is equal to the time rate of change of the kinetic energy of the jet, as follows:

$$P_j = \frac{1}{2} \cdot \dot{m} \cdot {v_j}^2 = \frac{1}{2} \cdot \rho \cdot A_j \cdot v_j(t)^3 \tag{E4}$$

The jetting power can also be expressed as follows:

$$P_j = \frac{1}{2\sqrt{2}} \cdot \pi \cdot {d_j}^2 (\rho)^{-\frac{1}{2}} \cdot (p_{in})^{\frac{3}{2}} \tag{E5}$$

This expression is useful for predicting the theoretical output jet power given an ampule pressure and nozzle diameter.

Linear regressions were used to generate a best fit line and 99% prediction regions of pressure-factor for each nozzle diameter (Supplementary figure 1 and Supplementary table 1). Subsequently, these regressions are reported for the corrected ampule pressure values in the *ex vivo* section.

Linear regression analysis also revealed a significant correlation between initial overshoot percentage and ampule pressure across all nozzles (Supplementary figure 2 and Supplementary table 2). Furthermore, we observed a relatively large number of outliers (11 out of 88 trials) in the overshoot values across all nozzles. These results are consistent with the numerical model of Symons which suggests a positive relationship between inertial loading and initial peaking, and that minor variations in void size due to bubbles can lead to large variations in initial peaking[21]. It is important to track initial overshoot as it has been demonstrated to have an impact on delivery characteristics in soft tissue[22]. Further characterisation should be performed to determine whether the overshoot we measured had a significant impact on subsequent *ex vivo* studies.

The high viscosity of biologics has posed a challenge to delivery in prior jetting systems[23]. As a point of reference, the viscosity of certain monoclonal antibody formulations exceeds 100 cP[24]. Bearing this in mind, we fixed the nozzle at 257 μm and the ampule pressure at 14.1 Bar, then varied the viscosity of the payload fluid by mixing glycerol with deionized water. Concentrations of glycerol ranged between 0% and 90% by weight, corresponding to viscosities between 1 and 219 cP[25]. We found that over this range of viscosities the system pressure-factor dropped significantly – from a mean of 0.82 to a mean of 0.40 (Extended data fig. 2h). We also performed a brief check of the fluid which we would later use for *ex vivo*

tests and found no significant changes in performance at three different input pressures (Extended data fig. 2i,j).

2. Data collection methods for characterisation of jetting apparatus

A diagram of our jetting apparatus is visible in Extended data fig. 1b. The main components of this apparatus were a hand-held jetting device for generating jets, and a force transducer for measuring jetting impingement force.

We designed the apparatus so that it was possible to quickly remove the hand-held jetting device via loosening of a clamp. The device was removed between each trial to access the ampule for refilling or to change nozzles. We refilled the ampule by disconnecting it from the main device assembly, submerging it in the payload fluid and then drawing its piston backward with a custom tool. An ampule volume of 232 µL was used for all experiments. Unless otherwise noted, 100% deionised water was used for all tests. We used a custom spanner bit to tighten and loosen nozzles when needed – the nozzle was threaded directly into the forward end of the ampule. Nozzles were smooth bell-shaped reducers, each with an entry diameter of 2 mm. The exit nozzle orifice diameters we used were 167, 257, 332, 446 and 551 µm.

Load was applied to the ampule by means of a pneumatic cylinder, which was connected to a compressed air supply with a digital pressure regulator. The triggering mechanism was activated by translation of a sleeve, which freed a set of radially positioned ball detents. These ball detents prevented a rod in the center of the device from translating. Once the detents were freed and the rod was released, pressure from the air cylinder was translated

directly to the ampule via the rod. This triggering mechanism was designed to closely approximate the sudden-release characteristic of our proof-of-concept device prototypes.

We used a 9215A piezoelectric force transducer and a 5165A4KH10 LabAmp® Dynamic Laboratory Charge Amplifier (Kistler Instrument, Amherst, NY, USA) to collect force data. The amplifier was connected to a Windows 10 laptop via Ethernet cable and data acquisition was triggered via Kistler's web-browser based interface. The total acquisition time used for all trials was 2 sec at a sampling frequency of 200 kHz. The jetting device was triggered by hand immediately after the data acquisition began. A subset of force profiles measured by the force transducer with a 257 μm nozzle can be seen in Extended data fig. 1c.

3. Analysis of data from jetting apparatus

Each dataset, containing 400,000 individual time points, was imported into MATLAB (MathWorks, Natick, MA, USA). Datasets often contained a large bias, which was removed by subtracting the average of the first 100,000 data points (prior to triggering) from the entire set. We also observed that many data sets contained large oscillations (at approximately 35 kHz), which we assumed to be instrument noise as their peaks swung into both the positive and negative sensing range. We removed this noise by applying a moving average filter with a half-width of ten time points (50 μsec) to each data set.

After filtering, we extracted the maximum load reading in each data set (assumed to be the peak output force, $F_{j,pk}$). Then, we marked the point at which jetting began by scanning for the earliest time when the measured force exceeded 10% of the extracted peak. Similarly, we marked the time that jetting ended as the point when the measured force dropped back below the same 10% threshold. The experimental jetting period, T_{exp}, is the difference between the

start and end point. A summary of jetting period results from our apparatus can be found in Extended data fig. 2a.

The experimental force profiles are not perfectly flat due to start-up and shut-down transients as well as sensor drift. To account for this, we considered the experimental steady-state force, $F_{j,exp}$, the mean of the section of the data that was observed to be most qualitatively flat. For the 167 μm nozzle, which had relatively long periods, the range of stable data points was $0.2T_{exp} < t < 0.4T_{exp}$. For all other nozzles, the stable range was $0.6T_{exp} < t < 0.8T_{exp}$. A summary of steady-state force measurements from our apparatus can be found in Extended data fig. 2b.

We define 'Overshoot' as the fraction by which the peak force exceeds the steady-state force:

$$Overshoot = \left(\frac{F_{j,pk}}{F_{j,exp}} - 1\right) \tag{E6}$$

Certain studies have shown that initial peaking in jet force can have an impact on performance[22], and therefore it must be controlled. A summary of peak force measurements and overshoot results from our apparatus can be found in Extended data fig. 2d,e.

Given an experimental steady-state jetting force measurement, $F_{j,exp}$, the experimental steady-state power output, $P_{j,exp}$, can be determined with the following equation:

$$P_{j,exp} = \frac{1}{2}\left(\rho A_j\right)^{-\frac{1}{2}}\left(F_{j,exp}\right)^{\frac{3}{2}} \tag{E7}$$

Previous research has posited that this quantity is correlated to delivery performance in skin tissue[14]. A summary of steady-state jetting power results from our apparatus can be found in Extended data fig. 2f.

Finally, to characterise the impact of our testing apparatus on jetting performance, we calculate the ratio of the experimental steady-state jetting force output, $F_{j,exp}$, to the theoretical value, F_j. We call the resulting quantity the 'pressure factor', η:

$$\eta = \frac{F_{j,exp}}{F_j} \tag{E8}$$

η can be multiplied with the experimental input pressure to determine the virtual or "corrected" ampule pressure, p_{in}', as follows:

$$p_{in}{}' = \eta \cdot p_{in} \tag{E9}$$

p_{in}' represents the ampule pressure of a lossless system that would result in the same force output as our experimental apparatus. We employ this correction to decouple reported ampule pressures from our experimental apparatus. In all our *ex vivo* results, we note p_{in}' ($\eta \cdot p_{in}$) as the control variable, rather than p_{in}. A summary of pressure factors for our apparatus can be found in Extended data fig. 2g.

We calculated the statistical uncertainty for overshoot and pressure factor with the linear regression function in GraphPad Prism (GraphPad Software, San Diego, CA, USA). Specifically, we generated best-fit lines and 99% prediction regions for the plots in Extended data fig. 2e,g. The regressions for pressure factor were later used to interpolate corrected ampule pressure for all *ex vivo* tissue studies. The results of these linear regressions can be found in Supplementary figures 1 and 2 and Supplementary tables 1 and 2.

4. Processing of volumetric data from *ex vivo* injection studies

Acquisition and reconstruction of these images were performed with Phoenix Datos|x (GE Measurement & Control, Billerica, MA, USA). This software reconstructs angular images into a set of virtual 2D slices. When combined, these slices form a 3D matrix of voxels with intensity values. In each 2D slice, high-intensity voxels are associated with less light

transmission and, in this case, correspond to parts of the sample where light is absorbed by the injected contrast agent. It was important to establish a threshold above which voxels would be considered to represent injected fluid. To achieve this, we used VGStudio MAX (Volume Graphics, Heidelberg, Germany) to designate a single "reference" slice in each file, manually selecting one region which was clearly tissue and one which was clearly contrast agent and averaging these two points' intensities. We set this value as the threshold for the entire matrix of voxels. Examples of processed scans are shown in Fig. 3a and Extended data figs. 3f and 4.

Again using VGStudio MAX, we segmented regions of the contrast fluid that were on top of, beneath and contained inside the tissue. To accomplish this, we opened the reference slice and manually enclosed the volume of fluid inside the tissue within a polygonal region of interest (ROI). We then examined the rest of the 2D slices and, if necessary, altered the size of the original ROI to enclose all relevant voxels. The ROIs on each slice were then combined to create a 3D (prismatic) ROI, thereby isolating all contrast fluid voxels contained inside the tissue. A similar procedure was then applied to the contrast fluid on top of and beneath the tissue, each time subtracting previously segmented voxels to prevent double sampling. Once segmented, the exact volume of each segment was calculated by summing all contrast fluid voxels contained within the 3D ROIs. Voxels were assigned a size of 10.65 pL in accordance with the micro-CT machine's predefined optical resolution (22 μm). A simplified representation of the segmentation process is shown in Extended data fig. 3f.

As was calculated in our volumetric diffusion study, the maximum diffusion rate of dye within the tissue is 0.67% per min (Extended data fig. 3g). Given the maximum amount of time allowed between injection and scan (15 min), the largest amount the volume could have

diffused is 10%. Thus, to generate a conservative estimate of the volume inside the tissue, we normalised each measurement of fluid in the tissue by a factor of 1.10. After this correction was performed, we calculated VDE of the sample by dividing the volume inside the tissue by the volume expelled from the ampule (232 μL). A standard Student's *t*-distribution was used to determine 95% confidence intervals for the submucosal volume and VDE. Statistical analysis was applied only after all other quantitative analyses – including segmentation and diffusion correction – were complete.

Although we were able to segment the injected fluid into three different categories (above, below and inside the tissue), we could only confidently account for volume that was inside the tissue. This is because, as noted previously, fluid on the tissue surface was often removed after the injection but before scanning. Additionally, contrast agent beneath the tissue would diffuse inconsistently into the foam mounting block. As a result, the total volume measured in the scan was, in most cases, either greater than or less than the volume originally expelled from the device. Considering this fact, we implemented a simple semantic filter in MATLAB to create a final accounting of where volume resided. Our semantic filter utilised the following steps:

1. Mark all volume inside of the tissue as 'submucosal'.
2. Calculate unaccounted-for volume by subtracting the submucosal volume from the volume expelled from the ampule (232 μL).
    a. Mark the unaccounted-for volume as 'intraperitoneal' if at least 20 μL was detected beneath the tissue during segmentation.
    b. Mark the unaccounted-for volume as 'luminal' if less than 20 μL was detected beneath the tissue during segmentation.

Given the binary nature of our semantic filter, we were not able to create a perfect account of volume. Namely, in some cases unaccounted-for volume was marked as purely 'intraperitoneal' even though contrast fluid was present both above and beneath the tissue. As such, the reader should be aware that in reported results, if volume is marked as 'intraperitoneal' this is an estimate of the result rather than a precise account.

Results from the above analyses are reflected in multiple parts, including Fig. 3, Extended data figs. 3 and 4, and in Supplementary tables 3 to 5.

5. Operation, design and assembly of the radial endoscopic device prototype (MiDe$_{RadEndo}$)

Our radial endoscopic prototype (MiDe$_{RadEndo}$) can deploy a therapeutic dose to the small intestine with assistance from an endoscope. Assembly of MiDe$_{RadEndo}$ is nearly identical to that of the autonomous version (MiDe$_{RadAuto}$), which is described in the next section. The one difference between the two devices is the assembly of the triggering mechanism. For the endoscopic version, this is accomplished by manually resetting the position of the ball detent and trigger pin. The device is triggered via a pneumatic line that is fed through an endoscope and connected to a PUN-3x0.5-SI fitting (Festo, Esslingen am Neckar, Germany) threaded to a CJPB4-5 pneumatic cylinder (SMC, Tokyo, Japan) on the device's body. When the pneumatic line is pressurised to 8 Bar, the cylinder releases a detent mechanism, allowing the main spring to apply force to the ampule via the main piston.

For ensuring tissue proximity during the endoscopic procedure, bags were prepared by welding two layers of BioBag 91116 thin films 30 µm thick (BioBag Zenzo, Hillerød, Denmark). Using a soldering iron heated to 200 C. After, a small hole was cut into the center

of the bag and glued (Araldite 2022-1; RS Components, Copenhagen, Denmark) on a 3D printed clip which was subsequently attached to the $MiDe_{RadEndo}$ device.

The nominal ampule volume and diameter for $MiDe_{RadEndo}$ are 188 µL and 6 mm, respectively. The stainless steel spring used in the device has an initial and final force of 51 and 9.2 N, respectively, resulting in theoretical ampule pressures between 14.7± 0.9 and 3.2± 0.5 Bar. The nozzle is oriented radially and its diameter is 240±10 µm (95% conf. n=3). The overall footprint of the device is 9 x 15 x 42 mm (excluding the pneumatic fitting,line, and bag), while the total high of the device including the bag was 35 mm. The device is made mostly of machined brass and other common metals. The piston seals are made from NBR-70. A section view is shown in Extended data fig. 6b.

6. Operation, design and assembly of the autonomous intestinal device prototype ($MiDe_{RadAuto}$)

The $MiDe_{RadAuto}$ is made of machined POM, PEEK and common metals. The piston seals are made from NBR-70. A photo of this device is shown in Fig. 1h and a section view is shown in Fig. 4f.

Assembly of $MiDe_{RadAuto}$ includes the following steps (Extended data fig. 6c-h):

PVA-pellet fabrication:

I. Cylindrical PVA-pellets with a 1.2 mm height and diameter are moulded in a custom stainless-steel mould (Kjul & Co, Brøndby, Denmark) using an AB 200 (AB Machinery, Quebec, Canada).
II. At a 5500 lbs clamp force, MOWIFLEX C 17 PVA (Kuraray, Hattersheim am Main, Germany) is injected at approximately 325 bar and held for 2 seconds.

III. PVA-pellets are removed from their support with a razor blade and visually inspected before use.

Main device assembly:

1. The ampule is filled with the payload fluid from the rear end.
2. Filling is stopped when the meniscus of the liquid reaches the rim of the ampule.
3. The piston is pushed into the ampule until its top surface is flushed with the rim. Meanwhile, excess fluid is drained through the nozzle.
4. The drive-rod and spring are slid into the case.
5. The compression fixture is attached to the case and cranked down until the spring is fully compressed.
6. The detent pin, followed by the PVA-pellet, are added to the rear end of the case, thereby fixing the spring in a compressed state.
7. The compression fixture is removed, and the ampule is joined with the case.

7. Operation, design and assembly of the autonomous gastric device prototype (MiDe$_{AxAuto}$)

MiDe$_{AxAuto}$'s bottom piece is machined from brass and its top piece from 7075 aluminium. The piston is made from POM, the seals from silicone rubber, and the burst-film from 25 µm-thick FEP. A photo of this device is shown in Fig. 1i and a section view is shown in Fig. 4i.

Assembly of MiDe$_{AxAuto}$ includes the following steps (Extended data fig. 7):

Potting the sugar-plug in the set-screw:

I. 5-10 chunks (approximately 1 mm in diameter) of solid isomalt (Sigma-Aldrich, St. Louis, MO, USA) are placed on a piece of aluminum foil.

II. A heat gun set to 645ºC is held 10 cm above the chunks for approximately 5 min or until the chunks have completely melted into a molten puddle and before visually significant caramelisation of the isomalt.

III. A pair of tweezers is used to quickly dip the set-screw in the puddle, which is then allowed to solidify (this takes an additional approximately 5 min).

IV. A damp cotton swab is used to polish away excess isomalt. It is then stored in desiccant until further use.

Main device assembly:

1. The bottom piece and piston are both lubricated and then pressed together.
2. The combined pieces are then screwed into the bottom half on the filling fixture.
3. The filling head is clamped against the device's nozzle orifice, and fluid is driven into the ampule. The piston displaces approximately 1.7 mm before topping-out.
4. The filling head is withdrawn and excess fluid is wiped away (not shown). The pre-lubricated burst film is then placed over the orifice followed by the setscrew which is then torqued with a custom spanner bit.
5. The filled bottom half is removed from the filling fixture and placed in a vice. Then, an approximately 3 mm diameter by 4 mm length dry ice pellet cylinder is placed in the piston.
6. The top piece is grasped by a custom tool and quickly fastened to the bottom, thereby sealing the gas chamber.

8. Force profile studies with MiDe$_{\text{RadAuto}}$ and MiDe$_{\text{AxAuto}}$

In the force profile studies (Extended data fig. 8), MiDe$_{\text{RadAuto}}$ was filled with deionised water and clamped so that its orifice was positioned directly above the transducer. In these trials, a stainless-steel rod that could be removed manually was used instead of the pellet to simplify

the triggering process. Four replicates were taken with $MiDe_{RadAuto}$. Data recording (100 kHz sampling rate) was started shortly before the activation pin was retracted.

The setup for testing the force profile of $MiDe_{AxAuto}$ was slightly different. Instead of clamping the device, we allowed it to sit on a plastic film with a 3 mm hole centered directly above the transducer. A pipette was used to apply a small puddle of water at the base of the device. This puddle activated the trigger through capillary action. Three separate $MiDe_{AxAuto}$s were tested with two or three replicates each. Data recording (50 kHz sampling rate) was started shortly before the puddle of water was applied, and data was later abridged to isolate the expulsion event.

9. Triggering studies with $MiDe_{RadAuto}$ and $MiDe_{AxAuto}$

The amount of time required for the $MiDe_{RadAuto}$ sugar-pellet to activate was determined by placing the assembled device filled with an aqueous green dye in a 50 mL tube filled with FaSSIF simulated intestinal fluid (Biorelevant, London, UK). The tubes were mounted in a HIM20 hybridisation oven (Boekel Scientific, Feasterville-Trevose, PA, USA) heated to 37ºC, and rotated at 45 rpm. When activation was observed, the time was noted. The activation period for $MiDe_{RadAuto}$ was measured to be 61.7±5.31 min (95% conf., n=3).

For $MiDe_{AxAuto}$, an SC2 high-speed camera (Edgertronic; Sanstreak, Campbell, CA, USA) was used to capture the triggering event and resulting jet. The frame rate used was 20,000 Hz. During these tests, the device was placed on a plastic film strewn across the rim of a glass beaker. This film had a 3 mm diameter hole in it on which the device orifice was center. The base of the device was then wetted with deionised water (dispensed by a pipette) and capillary action drew the water under the device causing the sugar plug to dissolve. When the

triggering was observed, the time was noted and the video buffer from 1.5 sec before and after the event was extracted.

The above process (with $MiDe_{AxAuto}$) was repeated 56 times in total. Of these replicates, 51 were successes. In two cases, the sugar plug ejected from the base of the device, likely indicating a compromised burst membrane. In three cases, the plug appears to have fractured, likely indicating a defect in the plug itself. We expect that such problems could be addressed with more robust quality control. The activation period was noted during 22 of the above trials and determined to be 240±54 sec (95% conf.). As will be noted later, the triggering time observed *in vivo* was significantly longer. A diagram of the setup is shown in Extended data fig. 9b. A triggering event is depicted in Fig. 1f. Results from this study are shown in Extended data fig. 9c.

10. Leakage study with $MiDe_{AxAuto}$

During assembly of $MiDe_{AxAuto}$ prototypes, each device is loaded with approximately 45 mg of solid CO_2. With this quantity of CO_2 at equilibrium, the 154 mm^3 chamber is saturated at 60 Bar with approximately 30 mg of gas-phase and 15 mg of liquid-phase CO_2. As the gas-phase diffuses through the rubber seal and out of the device, the liquid-phase boils off proportionally. Once the entirety of the liquid-phase CO_2 evaporates, the pressure in the chamber begins to drop.

Ideally, $MiDe_{AxAuto}$ would not leak at all, but achieving such hermeticity would require metallic diffusion barriers or welds, both of which we considered beyond the scope of our work. For our purposes, we simply needed to confirm that the leakage rate was slow enough that the buffer of liquid-phase CO_2 lasts long enough to perform experiments with the device.

To that end, we measured the mass loss of five devices using a digital scale with milligram-precision. These tests yielded a maximum leakage rate of 11.5±0.7 mg/hour (Extended data fig. 9d,e) which would result in a 1.3-hour period between the time the device was assembled and the time that pressure loss begins.

11. *In vivo* evaluation of GI transit and device activation after peroral dosing in Beagle dogs

Six male Beagle dogs weighing 9-13 kg were used to assess the GI passage and activation of a scaled-MiDe$_{RadAuto}$ device after peroral administration. Each animal was pretreated with subcutaneous pentagastrin (4.0 µg/kg) 30 minutes prior to dosing to ensure low gastric pH. The cap of a 00el enteric capsule (Lonza, Basel, Switzerland) was affixed to the rear of a loaded scaled-MiDe$_{RadAuto}$ with cyanoacrylate glue (Loctite, Westlake, OH, USA) to protect the PVA-pellet from liquid ingress. The rear end of the assembly was then encapsulated in half of a 000-gelatin capsule (Lonza) to protect the enteric capsule during handling and delivery of the device. When perorally dosing a device, the animal was also co-administered 5 mL of liquid, usually water, to entice swallowing. For this study it was important to preserve the low pH of the gastric cavity as to not compromise the performance of the enteric capsule, for which co-administration of neutral pH water was insufficient. Instead, a panel of juices were validated for co-administration via *in vitro* screening. Due to animal preference, apple juice (Harboe, Skælskør, Denmark) of pH 3.7 was used.

After dosing, each animal was imaged at 15-min intervals via fluoroscopy using a Ziehm SOLO FD C-arm (Ziehm Imaging, Nuremberg, Germany) to assess the activation state of the device. Once activation was confirmed, as seen by an extended spring in the device during fluoroscopy, the animal was perorally dosed 5 mL of 320 mg iodine/mL Visipaque (GE

HealthCare Technologies, Chicago, IL, USA) contrast agent, diluted to 30% by volume with apple juice, to deduce the localization of the device. The contrast agent would highlight the gastric cavity and the juxtaposition of the gastric cavity to the device would denote whether the device had activated in the gastric cavity or the small intestine. If the device was confirmed to have activated in the gastric cavity no sampling was performed. If the device was inferred to have activated in the small intestine, blood was sampled at 2 and 4 hours to assess systemic exposure in a binary fashion. Each animal was monitored daily via fluoroscopy to verify device passage. The results of the transit study can be seen in Extended data fig. 10j,k.

12. Device passage studies

Passage studies were performed with $MiDe_{AxAuto}$s to confirm that they would safely exit the digestive system. In total, three animals were used, each passing a total of three devices. The devices we used were “dummies”. Dummy devices were designed to have identical external geometry and mass distribution as the actual devices and had a radio-opaque (stainless steel) bottom and plastic top. They neither contained a therapeutic nor were pressurised.

The animals were sedated, and devices were deployed in a similar manner to non-terminal pharmacokinetic studies. For the first week after deployment, pigs were X-rayed daily to observe device transit. During the subsequent weeks, pigs were X-rayed every other day. Pine shavings from the animals’ enclosures were collected in large garbage bags and X-rayed on a weekly basis to check for devices. Meanwhile the animals’ behaviour and eating habits were observed.

We were able to locate all three devices from two pigs and two devices from one pig. However, X-rays showed that all devices were passed by the end of the third week, so the missing device was likely lost during cage cleaning. Furthermore, all pigs were behaving and eating normally throughout the duration of the study. Images of the devices in transit can be found in Extended data fig. 10a-f.

13. Inflammation study

Inflammation studies were performed with $MiDe_{AxAuto}$s to assess whether jets caused significant inflammation at or around the injection site. We deployed five fully pressurised $MiDe_{AxAuto}$s filled with deionised water to the stomach of one pig. The animal was sedated, and devices were deployed and retrieved in a similar manner to non-terminal pharmacokinetic studies. Then, 24 hours after the deployment, the animal was re-anaesthetised and euthanised. Immediately following euthanasia, a laparotomy was performed and the entire stomach was harvested (using the previously described organ extraction methods). Then, tissue from the area of the stomach where the devices were deployed was carefully excised. A section of control tissue was also extracted from the opposite side of the stomach.

The main tissue section was then cut into five pieces and placed in standard histology cassettes. The section of control tissue was similarly cut into three pieces and placed in cassettes. The tissue samples were then fixed in 10% buffered formalin for no less than 24 hours, then transferred to 70% ethanol for storage. From there, the samples in each cassette were further sectioned, mounted, stained and imaged using similar histology methods to those described in the *ex vivo* injection studies (see Methods).

In total, 88 slices (5 µm thick) from the area of interest were imaged. Careful visual inspection of the results revealed no histopathological changes that might have been caused by the jet injections. No tissue damage to the mucosa or submucosa, no haemorrhaging and no major inflammatory reactions were observed. We were able to identify one acute submucosal inflammatory focus with a dominance of polymorph nuclear cells that was approximately 1 mm in length. However, no overlying tissue damage was observed in that region.

The results from this inflammation study are provided in Extended data fig. 10g. Also, two images of gastric tissue with penetration wounds from the previous *ex vivo* injections are provided in Extended data fig. 10h,i for comparison.

14. Human insulin and insulin analogue assay

The AlphaLISA® assay (Perkin Elmer, Shelton, CT, USA) employs two monoclonal antibodies against insulin sandwiched between an acceptor bead and a donor bead, which emit light proportional to insulin concentration when excited.

Before the assay was initiated, one liter of luminescent oxygen channeling immunoassay (LOCI) buffer solution containing the following proportions of reagents was prepared for general dilution needs: 25 mM HEPES, 50 mM NaCl, 10 mM K-EDTA, 2 mg/mL Dextran, 0.5% BSA, 0.1% BGG, 0.1% Tween-20, 0.01% Proclin 300, 0.01% Gentamycin and 0.2 mg/mL HBR1 – pH 7.4. In addition to formulating the LOCI buffer, AlphaLISA® acceptor beads were pre-conjugated with HUI-018 (Novo Nordisk) for capture of human insulin and NN454-1F31 (Novo Nordisk) for capture of insulin analogue to the acceptor beads, at 5 mg/mL stock concentration. Biotinylated 4F1 (Novo Nordisk) antibody solution

was prepared at a stock concentration of 4.86 µM for detection of human insulin, and biotinylated S1 (Novo Nordisk, Denmark) antibody solution at a stock concentration of 3.89 µM for detection of insulin analogue. Streptavidin coated AlphaLISA® donor beads were purchased from Perkin Elmer at a stock concentration of 5 mg/mL.

An eight-point standard curve (including one blank) was prepared by spiking human insulin into analyte-free porcine serum at concentrations between 20.6 and 15,000 pM. 2 µL of each standard point and samples from experimental time points were stamped into a Corning 384-well plate (Corning, Corning, NY, USA) with one duplicate each. Stamping was performed with a Tecan liquid handling deck (Tecan, Männedorf, Switzerland). The sample was mixed with 15 µL of conjugated acceptor beads and biotinylated antibody mixture, diluted in LOCI buffer to 33.3 µg/mL of acceptor beads and 9 nM biotinylated antibody for human insulin and 4 nM for insulin analogue, respectively.

The plate was then struck, covered and incubated for 60 min at room temperature. After the incubation, 30 µL of streptavidin-coated donor beads diluted with LOCI buffer to 66.7 µg/mL was added to each well under green filtered light. The plate was again struck, covered and incubated for 30 min at room temperature. Finally, the plates were read on a Tecan M1000 Plate Reader (Tecan) at a filter bandwidth of 520–645 nm, and an excitation wavelength of 680 nm. Total measurement time per well was 210 ms, including a 70 ms excitation time.

Data analysis was performed in GraphPad Prism using a log(agonist) vs. response, variable slope, four-parameter sigmoidal model for insulin quantitation. Once concentrations for each individual study were obtained, they were plotted against their respective time points, as shown in Fig. 4a,b,g,j.

15. Quantification of siRNA

The quantification of siRNA in plasma samples was conducted employing the Meso Scale Discovery (MSD) platform. Custom-designed biotinylated detection and digoxigenin-labeled capture probes (Integrated DNA Technologies, Coralville, IA, USA), complementary to the sequence of siRNA, were diluted in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and employed for the specific detection of siRNA. To prevent coagulation during heating, plasma samples were diluted in 1X PBS, 1% Tween solution. Subsequently, 250 µL of the capture and detection probe working solution (6X SSPE Tween-20, capture probe at 20 nM and detection probe at 2.5 nM) was added to micronic plate wells containing the diluted samples and thoroughly mixed. Following this, 100 µL of this mixture was transferred into a 96-well PCR plate (AB0600L; Thermo Fisher Scientific, Waltham, MA, USA) and subjected to incubation in a thermocycler (Applied Biosystems™ Veriti™ Dx 96-well Thermal Cycler; Thermo Fisher Scientific) programmed as follows: initially at 95°C for 5 min, followed by incubation at 25°C for 30 min, and then holding at approximately 10 min at 12°C.

Prior to transferring the samples to the 96-well MSD plate (MSD GOLD 96-well Small Spot Streptavidin SECTOR Plate; Meso Scale Diagnostics, Rockville, MD, USA), the plate underwent blocking by incubation on a shaker plate using a blocking buffer (Synthetic Blocking buffer ELISA; Kementec Solutions, Taastrup, Denmark), followed by triple washing with PBS, Tween-20. After completion of the hybridization process in the thermal cycler, 25 µL aliquots from each sample were transferred onto the pre-blocked MSD plate, where they underwent 1 h incubation at room temperature facilitated through frequent shaking utilizing a shaker-equipped plater. Following, additional washing steps were performed, after which the binding of the siRNA molecules was identified through 1 h incubation with a sulfo-tagged antibody specifically binding to the digoxigenin component of

the detection probe. Subsequent to another round of washing, 150 µL of 1X Read Buffer A was added to each well of the MSD plate, followed by the detection of the signal using the SECTOR Imager 3000. A standard curve was constructed using known concentrations of siRNA to determine the concentration in the plasma samples.