Peer Review File

Manuscript Title: Cephalopod-inspired ingestible jetting devices for drug delivery

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

The authors have completed comprehensive development and analysis of the performance of a new set of drug delivery tools, namely tethered or untethered microjet devices.
As highlighted by the authors, while the concept of using a microjet device in the buccal cavity has been considered before, here, it is cleverly and uniquely combined with prior work on ingestible deployable devices.
The significance of this is highlighted by the bioavailability achieved with insulin and GLP-1 - at least an order of magnitude above that obtainable with current oral pills.
The data is presented clearly. The authors have developed some novel experimental techniques and corresponding new modes of quantification are introduced (e.g. Volumetric delivery efficiency).
References are appropriate and the paper is well written.

Suggested Improvements:
While the bioavailability achieved is impressive, the route cause of the high variablity is not fully substantiated. Biological variance is referenced. I would have expected to see some modified Franz Cell experiments ex vivo to track more accurately, where does the dye/payload go in untethered and tethered device formations, to try and decipher more thoroughly how much of the variance is due to the substrate or device performance.
I suggest that the bioavailability achieved is highlighted clearly in Figure 4 or tabulated separately.
I find it confusing that the muscularis is recognised separately in Extended Data Fig. 5, but in Extended Data Fig. 4 classification is either luminal, submucosal or IP.
In Fig.1, rather than including coins for comparison, size 00 or 000 capsules, that are conventionally swallowed would be a more useful visual comparitor.

Referee #2 (Remarks to the Author):

This manuscript focuses on developing ingestible devices that can generate high pressure jets in different regions of the gastrointestinal tract. Two classes of devices are developed, axial devices and radial devices. Axial devices are suitable for organs like the stomach and radial devices are suitable for organs like the small intestine. Two modes of delivery are investigated, tethered systems, which will be delivered via an endoscopic catheter and autonomous systems, which will be ingested orally. In vivo experiments are performed in swine and canines, where the devices were inserted into the gastrointestinal tract and triggered at various pressures and the systemic absorption of insulin and GLP-1 was determined. From these studies the authors determined the minimum pressure needed for efficient absorption. In vitro experiments were further done on a variety of different tissue samples.
This is the first study investigating the absorption of biomolecules after delivery via a jet of fluid and has the potential to have a significant impact on the field of drug delivery. The devices developed in this manuscript are also innovative and represent significant advances to the field. Additional clarification of concepts is needed to make the manuscript accessible to a broad audience. These are listed below.

(1) Very little information is provided about the devices in the main text. The new devices are the major development in the paper and they need to be described in the main text. A comparison of their device design to other pressure driven flow devices should be made, to put the devices in perspective. The innovations made in the device design and fabrication need to be highlighted. It is also unclear how many devices were made.

(2) It is unclear why cephalopods are being discussed, and what the relevance to their devices is.

(3) The flow rate of the fluid being ejected from the devices needs to be measured and correlated with absorption. This information will be very helpful to other researchers trying to develop their own devices.

(4) Some information about the size limits of absorption after jet delivery would strengthen the paper. The absorption of a protein larger than insulin should be measured to get a feel for the mechanism of absorption. If the jet is penetrating the tissue, large proteins should also get absorbed.

Niren Murthy

Referee #3 (Remarks to the Author):

Recommendation: Minor revisions needed.

1. General comments:
The authors report on the development and application of some microjet devices (MiDe) for drug delivery in the gastrointestinal (GI) tract. Following a biomimetic approach inspired by cephalopods, they invented MiDes capable of axial and radial jet delivery in various conditions within the GI tract.

This is a comprehensive and thorough study encompassing the prototyping and characterization of novel MiDes. Additionally, it involves the evaluation of the designed MiDes through in vitro and ex vivo studies, as well as in vivo trials conducted on large animal models such as swine and dogs. The authors also compared the effectiveness of MiDe devices with traditional delivery methods and demonstrated promising results achieving double-digit bioavailability for oral biologics through jetting.

In summary, this manuscript represents an innovative approach, a rigorous methodology and a significant contribution to the field of drug delivery. Overall, the paper is well written and the data well-presented but there are some questions, which need to be addressed. Therefore, I recommend publishing this manuscript with minor revisions in Nature.

2. Major concerns:

Page 7 Line 141-142: The authors should define the composition and provide some more details on the “sugar-plug” either in the main manuscript or in the supplementary data. In particular, it is not clear what exact mechanisms are used as trigger (probably humidity/pH?), how reproducible and at what time scale the jet mechanism is initiated after e.g. first contact with an aqueous environment. It also deserves some discussion, how stable this mechanism is during storage and unintended/premature exposure to water or humidity. In addition, it is unclear how the “sugar-plug” and the mentioned coating are interacting. The authors should explain the triggering mechanism of the devices in more detail in the manuscript or in the supplementary data.

Page 24 Line 455-460 & Page 47 Line 830-831: The authors state that the “polymer-pellet” is composed of “injection molding PVA”. However, neither the main manuscript nor the supplementary data specifies the particular properties of this specific polymer as used in this study (probably poly(vinylalcohol)?), or its manufacturer.

Page 7 Line 144-148: Might this brief pressurized release, encompassing both the release itself and the emitter propelling approximately 1 cm in the opposite direction, lasting only 1-2 milliseconds, pose any risk of harm or irritation to the patient? Particularly in the context of treating high-risk patients with commonly prescribed antirheumatic drugs, how do you assess the risk of gastric or duodenal ulcer perforation? Please compare and discuss the potential risks in the context of the AMUNO GITS/OSMOGIT scandal of 1983, where casualties had been reported and the system disappeared from the marked since.

Page 24 Line 455-460: Employing endoscopy, the authors placed the devices exactly/close to where there were supposed to release the drug. If administered orally, how can you ensure that the release occurs exclusively within the intestine, and moreover correctly oriented towards the mucosa? Could you please specify on the pH-dependant release mechanism trigger, especially considering how this system can prevent premature or delayed release based on the "food effect"?

Page 25 Line 472-481: Similar to the above question: Through endoscopy, you positioned the devices precisely in proximity to the designated drug release site within the stomach. If administered orally, how can you ensure the release is limited to the stomach as well as only occurring while correctly oriented towards the mucosa? Could you please specify on the pH-dependant release mechanism trigger, especially considering how this system can prevent premature or delayed release based on the "food effect"?

3. Minor concerns:

Page 7 Line 147-149: The authors explored the VDE up to 0.5 cm, but why not up to 1 cm? As the authors themselves mention, doing so could provide additional insights into the uncertainty.

Page 8 Line 163-165: At which injection pressure did the authors apply the GLP-1 analogue?

Page 17 Fig.4 a,b,c, and e: Data points at/after the 8h time-point are not visible or partially visible. Please correct the graph image accordingly.

Page 46-48 825-845: Given the intricate nature of this sophisticated system, how would you judge its overall biodegradability and sustainability? Furthermore, how would you evaluate its scalability for production on a broader scale to cater to a larger patient population?

Page 13 Fig.1f&g: The visibility of the letters f&g is significantly compromised by the whitish background. This issue could be addressed by changing it to black.

Page 13 Fig.1f: Incorporating a time and length scale would enhance the comprehension of the propulsion/expulsion phenomenon.

Reviewer: Professor Claus-Michael Lehr

Author Rebuttals to Initial Comments:

Point-by-point response for Manuscript 2023-09-16480

Editor

From our perspective, we are particularly keen to see strengthening of your manuscript to bring the work closer to being feasible for practical application. In particular, we feel that in vivo data would be strengthened through oral delivery of devices, rather than relying on accurate pre-positioning of devices through endoscopy. This point is specifically mentioned by referee 3.

RESPONSE: Thank you for your comments and the opportunity to address the feasibility aspects of our manuscript. We have taken into account your perspectives and have made significant strides to emphasize the practical application potential of our work, particularly in the context of in vivo data and the oral delivery of devices.

To enhance the feasibility of our work, we have specifically focused on the $MiDe_{RadAuto}$ system and conducted additional animal studies to address the crucial aspect of gastric transit. We have conducted additional in vivo experiments to investigate activation in the target location, specifically the intestine. These capsules are designed to remain intact in the acidic environment of the stomach and dissolve under neutral pH conditions in the intestine. The data from these experiments are now included in Extended data fig. 10j,k to support our claims, and we have additionally addressed this in the main body of the manuscript.

Although we would ideally have pursued oral dosing with pharmacokinetic studies; unfortunately, there is no suitable animal model that could adequately inform us on human outcomes in this context. We acknowledge the significance of oral dosing, however access to the pigs' small intestine can be achieved meaningfully by utilizing endoscopy. The devices were dropped from the endoscopic holder, without fixation or specific orientation towards the tissue, allowing them to activate autonomously in the fully awake minipigs due to the short anesthesia period.

We believe that our efforts have significantly enhanced the practical application potential of our work, and we are committed to continuously refining our methodologies to address the feasibility concerns you have raised.

Thank you for your insightful feedback, and we are dedicated to further strengthening the practical application aspects of our research.

Referee 1 Comments

The authors have completed comprehensive development and analysis of the performance of a new set of drug delivery tools, namely tethered or untethered microjet devices.
As highlighted by the authors, while the concept of using a microjet device in the buccal cavity has been considered before, here, it is cleverly and uniquely combined with prior work on ingestible deployable devices.
The significance of this is highlighted by the bioavailability achieved with insulin and GLP-1 - at least an order of magnitude above that obtainable with current oral pills.
The data is presented clearly. The authors have developed some novel experimental techniques and corresponding new modes of quantification are introduced (e.g. Volumetric delivery efficiency). References are appropriate and the paper is well written.
RESPONSE: Thank you very much for your thorough assessment and review of our manuscript, including the very positive feedback and the constructive suggestions for improvement.

Suggested Improvements:
While the bioavailability achieved is impressive, the route cause of the high variablity is not fully substantiated. Biological variance is referenced. I would have expected to see some modified Franz Cell experiments ex vivo to track more accurately, where does the dye/payload go in untethered and tethered device formations, to try and decipher more thoroughly how much of the variance is due to the substrate or device performance.
RESPONSE: There are three potential sources of pharmacokinetic variation: the mechanics of the jet, biological variation, and jet positioning prior to delivery. Our study assessed each of these factors in vitro with device jet characterization (Extended data figs. 1, 2 and 8 – steady state jet forces had coefficients of variation [CVs] <10%), ex vivo variation by volume delivery efficiency (VDE) (Fig. 4b,c – VDE CVs of 21% and 44% for intestine and stomach, respectively), and the impact of jet positioning to the tissue on VDE (Fig. 3h,i - VDE loss of up to 40% at a 45 degree angle), respectively. We have now included a paragraph in the Discussion (Pages 10-11, Lines 270-291) to describe which sources drive pharmacokinetic variability.

I suggest that the bioavailability achieved is highlighted clearly in Figure 4 or tabulated separately.
RESPONSE: Thank you for your valuable suggestion. We agree that highlighting the achieved bioavailability would provide a clearer understanding of our results. Accordingly, we have now incorporated your recommendation and included a tabulation in Fig. 4e to clearly display the bioavailability achieved. We appreciate your feedback and believe these changes will enhance the readability and comprehension of our findings.

I find it confusing that the muscularis is recognised separately in Extended Data Fig. 5, but in Extended Data Fig. 4 classification is either luminal, submucosal or IP.
RESPONSE: Thank you for your observation. The difference in classification between Extended data figs. 4 and 5 is indeed a result of the distinct capabilities of the imaging techniques applied. CT scanning, used in Extended data fig. 4, is excellent for measuring depot size but struggles to resolve individual tissue layers, i.e. muscularis. Conversely, histological staining and microscopy techniques, applied in Extended data fig. 5, excel at distinguishing different tissue layers. However, the latter techniques require processing steps, such as fixation, which prevent them from providing information regarding volume (VOE). In an effort to provide the most comprehensive information, we chose to include the muscularis in Extended data fig. 5. We hope this clarifies your concern and appreciate your attention to detail.

In Fig.1, rather than including coins for comparison, size 00 or 000 capsules, that are conventionally swallowed would be a more useful visual comparitor.
RESPONSE: Thank you for your suggestion. We have updated Fig. 1h,i to include size 000 capsules as comparators, which better reflect the conventional objects that are swallowed.

Referee 2 Comments

This manuscript focuses on developing ingestible devices that can generate high pressure jets in different regions of the gastrointestinal tract. Two classes of devices are developed, axial devices and radial devices. Axial devices are suitable for organs like the stomach and radial devices are suitable for organs like the small intestine. Two modes of delivery are investigated, tethered systems, which will be delivered via an endoscopic catheter and autonomous systems, which will be ingested orally. In vivo experiments are performed in swine and canines, where the devices were inserted into the gastrointestinal tract and triggered at various pressures and the systemic absorption of insulin and GLP-1 was determined. From these studies the authors determined the minimum pressure needed for efficient absorption. In vitro experiments were further done on a variety of different tissue samples.

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RESPONSE: Thank you very much for your thorough assessment and review of our manuscript, including the very positive feedback and constructive suggestions for improvement.

(1) Very little information is provided about the devices in the main text. The new devices are the major development in the paper and they need to be described in the main text. A comparison of their device design to other pressure driven flow devices should be made, to put the devices in perspective. The innovations made in the device design and fabrication need to be highlighted. It is also unclear how many devices were made.

RESPONSE: In response to your feedback, we have enhanced the manuscript's text to explain the reasoning more comprehensively behind our device's design and have clarified the number of designs developed specifically for this study (page 7, lines 169-177).

(2) It is unclear why cephalopods are being discussed, and what the relevance to their devices is.

RESPONSE: We have elaborately restructured our explanation on how the unique locomotion and directional control capabilities of cephalopods have inspired the design principles of our devices (page 3, lines 66-72). We have emphasized that we drew inspiration from cephalopods to design different Microjet Device (MiDe) systems, each with a unique, predetermined jet orientation. This allows our devices to cater to diverse conditions within the gastrointestinal (GI) tract with improved precision.

(3) The flow rate of the fluid being ejected from the devices needs to be measured and correlated with absorption. This information will be very helpful to other researchers trying to develop their own devices.

RESPONSE: We appreciate your insightful suggestion regarding the measurement of the fluid flow rate. From the Bernoulli energy balance equation (Supplementary information - Methods, E3), the jet flow rate (Q) and jet velocity (V) can be directly calculated from the density of water (ρ), measured jet force (F), and the nozzle cross sectional area (A): $Q = v \cdot A = \sqrt{F \cdot A / \rho}$. To elucidate this relationship for the reader, the calculated steady-state flow rate has now been added in Fig. 4e and in Extended data fig. 2c.

(4) Some information about the size limits of absorption after jet delivery would strengthen the paper. The absorption of a protein larger than insulin should be measured to get a feel for the mechanism of absorption. If the jet is penetrating the tissue, large proteins should also get absorbed.

RESPONSE: We appreciate your valuable suggestion on investigating the absorption limits post-jet delivery. It is important to note that previous studies utilizing jetting systems, such as transdermal and sublingual jets, have demonstrated pharmacokinetics comparable to standard hypodermic injection (Hogan NC et al. Annu Int Conf IEEE Eng Med Biol Soc 2015;2015:7336-7340, doi: 10.1109/EMBC.2015.7320086 and Ref. 10). These studies provide a strong foundation for the effectiveness of jet delivery systems across a range of molecules, including proteins larger than insulin. Moreover, recent data from Oral Device (SOMA) research has underscored the capability of standard injections in the GI tract to effectively deliver monoclonal antibodies (new Ref. 14). In addition to these points, it is important to note that the injection location within the tissue is consistent between the SOMA device, where Humira (approximately 150 kDa) was dosed, and the location where we deposit via jet

injection. This consistency has been confirmed through histological analysis, providing evidence that the jet injection method targets the same tissue regions as the SOMA device. This alignment further supports the potential for effective absorption of larger molecules using jet injection technology.
Furthermore, we have conducted a study using siRNA to assess the bioavailability of a new compound (methods described on page 23, lines 613-615 and in the Supplementary information – Methods Sections 11 and 15). The data from this study, now included in Fig. 4d (described on page 8, lines 212-214 and discussed on page 11, lines 293-300), supports our findings and underscores the potential of our jet delivery system to effectively administer and absorb larger molecules . Notably, the new compound, at approximately 21 kDa, presents a significant size difference compared to insulin and GLP-1 analogues (approximately 4-6 kDa), further highlighting the good absorption of larger molecules via jet delivery systems (bioavailability of 82%). To complement our current findings, future work could expand on this aspect to delineate the absorption mechanisms further and establish a more comprehensive size-to-absorption correlation for effective delivery via this method.

Referee 3 Comments
Recommendation: Minor revisions needed.

1. General comments:
The authors report on the development and application of some microjet devices (MiDe) for drug delivery in the gastrointestinal (GI) tract. Following a biomimetic approach inspired by cephalopods, they invented MiDes capable of axial and radial jet delivery in various conditions within the GI tract.

This is a comprehensive and thorough study encompassing the prototyping and characterization of novel MiDes. Additionally, it involves the evaluation of the designed MiDes through in vitro and ex vivo studies, as well as in vivo trials conducted on large animal models such as swine and dogs.
The authors also compared the effectiveness of MiDe devices with traditional delivery methods and demonstrated promising results achieving double-digit bioavailability for oral biologics through jetting.

In summary, this manuscript represents an innovative approach, a rigorous methodology and a significant contribution to the field of drug delivery. Overall, the paper is well written and the data well-presented but there are some questions, which need to be addressed. Therefore, I recommend publishing this manuscript with minor revisions in Nature.
RESPONSE: Thank you very much for your thorough assessment and review of our manuscript, including the positive feedback and constructive suggestions for improvement.

2. Major concerns:
Page 7 Line 141-142: The authors should define the composition and provide some more details on the "sugar-plug" either in the main manuscript or in the supplementary data. In particular, it is not clear what exact mechanisms are used as trigger (probably humidity/pH?), how reproducible and at what time scale the jet mechanism is initiated after e.g. first contact with an aqueous environment. It also deserves some discussion, how stable this mechanism is during storage and unintended/premature exposure to water or humidity. In addition, it is unclear how the "sugar-plug" and the mentioned coating are interacting. The authors should explain the triggering mechanism of the devices in more detail in the manuscript or in the supplementary data.
RESPONSE: We apologize for any confusion caused regarding the composition and dissolution of the "sugar-plug" utilized in our study.
The "sugar-plug" is composed of isomalt (Sigma, USA), a sugar substitute, which readily dissolves in water. During the manufacturing, the setscrew is dipped in the molten isomalt and is then allowed to solidify to create a solid "sugar-plug" within the setscrew. We would like to direct your attention to the Extended data fig. 7 and Supplementary information - Methods Section 7 of our manuscript, where we have detailed the manufacturing of the "sugar plug" used in this study.
The triggering mechanism indeed relies on the dissolution of the "sugar-plug" from moisture. When the "sugar-plug" comes in contact with a solvent it starts to dissolve. Upon complete dissolution, the burst-membrane ruptures which in turn releases the jet. We would like to kindly direct your attention to the Extended data fig. 9, where the triggering mechanism is illustrated in more detail. In Extended data fig. 9c, we present the ratio of successful dissolution experiments (disintegration) versus unsuccessful failure modes (fracture, ejection), and in Supplementary information – Methods Section 9, we describe the method and report mean triggering times of 61.7 min and 240 sec for $MiDe_{RadAuto}$ and $MiDe_{AxAuto}$, respectively. In the Discussion, we have now additionally addressed the importance of storage and further optimization for a marketed product (page 12, lines 318-321).

Page 24 Line 455-460 & Page 47 Line 830-831: The authors state that the "polymer-pellet" is composed of "injection molding PVA". However, neither the main manuscript nor the supplementary data specifies the particular properties of this specific polymer as used in this study (probably poly(vinylalcohol)?), or its manufacturer.
RESPONSE: We apologize for any confusion caused regarding the specific material and manufacturer of the "polymer-pellet" utilized in our study. The "polymer-pellet" is indeed composed of polyvinyl alcohol (PVA), specifically Mowiflex C 17 from Kuraray. We would like to kindly direct your attention to the Supplementary information - Methods Section 6 of our manuscript, where the type and source of the PVA used in this study are detailed.

Page 7 Line 144-148: Might this brief pressurized release, encompassing both the release itself and the emitter propelling approximately 1 cm in the opposite direction, lasting only 1-2 milliseconds, pose any risk of harm or irritation to the patient? Particularly in the context of treating high-risk patients with commonly prescribed antirheumatic drugs, how do you assess the risk of gastric or duodenal ulcer perforation? Please compare and discuss the potential risks in the context of the AMUNO GITS/OSMOGIT scandal of 1983, where casualties had been reported and the system disappeared from the marked since.
RESPONSE: We acknowledge your concerns regarding the potential risks associated with the brief pressurized release of our delivery system, especially in the context of high-risk patients being treated with antirheumatic medications. It is indeed critical to assess the safety profile of any new drug delivery method. Amuno is a NSAID with well-known gastrointestinal (GI) side effects. NSAID drug products delivered by OROS devices (osmotic-controlled release oral delivery system) have caused serious GI adverse events due to the extended exposure of the GI mucosa and, thus, they have been withdrawn from the market. Based on this previous experience, the administration of NSAID drug products with Oral Delivery devices is not considered viable and safe.
In contrast, our delivery system's brief interaction with the GI wall is not anticipated to pose significant safety concerns. The recoil force exerted by our oral delivery device on the GI wall is considerably lower than that associated with routine clinical procedures like endoscopy, which have a well-established safety record.

Page 24 Line 455-460: Employing endoscopy, the authors placed the devices exactly/close to where there were supposed to release the drug. If administered orally, how can you ensure that the release occurs exclusively within the intestine, and moreover correctly oriented towards the mucosa? Could you please specify on the pH-dependant release mechanism trigger, especially considering how this system can prevent premature or delayed release based on the "food effect"?
RESPONSE: We are grateful for your insightful queries regarding the targeted release and orientation of our oral delivery device within the GI tract. Please find our detailed response to each of your concerns below:

1) Release in target location (i.e. intestine): We agree that the correct placement of the device is crucial for successful injection into the submucosal space. To address this aspect and to demonstrate its feasibility, we have conducted additional in vivo experiments using MiDe$_{\text{RadAuto}}$ devices with a pH-sensitive capsule to protect the PVA activation pellet from ingression of stomach liquids. These commercial capsules are designed to remain intact in the acidic environment of the stomach and dissolve under neutral pH conditions in the intestine. We have now included the data from these experiments in Extended data fig. 10j,k to support our claims and additionally addressed this in the main body (pages 9-10, lines 242-259).

2) Food effect: We acknowledge that there is a statistical chance of premature activation of the device due to the food effect and patient-to-patient pH variation. However, we have tested the devices only in fasted animals, as stated in the manuscript. We would like to highlight that the enteric capsules we used are specifically designed to prevent premature or delayed dissolution. Furthermore, we are aware that the food effect can vary between individuals and that this could potentially impact the activation of the device. This is a limitation that we have now acknowledged in the manuscript. We believe that further studies are needed to address this issue and we have included this as a direction for future work in the Discussion (page 10, lines 267-268).

3) Correct orientation towards mucosa: We fully agree that the location and orientation of the device in the GI tract is of great importance for an orally administered device that functions in this manner, particularly during activation. The dimensions and form factor of the device ensure that the device both transits the GI in such a way that the nozzles are orthogonal to the epithelial lining of the lumen and, by nature of having 180 degree offset nozzles, the distance of at least one of the nozzles to the tissue is minimized without active interference. Such transit also ensures that the contact angle is as near-orthogonal as possible without active interference. These traits have been evidenced via systemic pharmacokinetic exposure of the loaded candidate API (Fig. 4g).

In summary, our responses, and the supplementary data aim to comprehensively address the issues raised regarding our device's function within the GI tract. We are dedicated to the ongoing refinement and thorough evaluation of our delivery system, and we look forward to further exploring these considerations.

Page 25 Line 472-481: Similar to the above question: Through endoscopy, you positioned the devices precisely in proximity to the designated drug release site within the stomach. If administered orally, how can you ensure the release is limited to the stomach as well as only occurring while correctly oriented towards the mucosa? Could you please specify on the pH-dependant release mechanism trigger, especially considering how this system can prevent premature or delayed release based on the "food effect"?
RESPONSE: Our device is designed with a specific shape and density distribution, allowing it to self-orient towards the mucosal lining of the stomach due to the density difference relative to the gastric fluid. This self-orientation concept was introduced in our previous publication (Ref. 1), which is cited in the subsequent paragraph relative to the paragraph that you refer the question to.
The self-orientation mechanism requires a certain amount of time to align the device towards the mucosal lining of the stomach after replacement, i.e. movement (see Ref. 1). Simultaneously, the isomalt sugar-plug is designed to dissolve at a predictable rate to ensure timely activation. These overlapping timeframes or synchronicity introduce a variable into the process, which can theoretically lead to instances where the device may not be perfectly aligned at the moment of activation. However, given the rapid self-orientation process, such instances would likely be infrequent thereby minimizing the likelihood of misalignment.
In our device's activation mechanism we use a sugar (isomalt) for the plug, which dissolves passively in the stomach. Isomalt dissolution is not pH-dependent, which means its dissolution rate remains consistent across a range of pH levels typically found in the stomach. This feature makes isomalt an ideal candidate for achieving a predictable release of the therapeutic dose, regardless of the varying gastric conditions.

3. Minor concerns:
Page 7 Line 147-149: The authors explored the VDE up to 0.5 cm, but why not up to 1 cm? As the authors themselves mention, doing so could provide additional insights into the uncertainty.
RESPONSE: We value the reviewer's point on the possibility of expanding our VDE exploration of the stand-off distance to 1 cm. We focused on a maximum of 0.5 cm as our analysis indicated that this range yielded sufficient data to draw robust conclusions. The increments chosen (0 cm, 0.25 cm, and 0.5 cm) allowed us to discern clear patterns and make statements about the uncertainty profile. By comparing three specific distances within this range, we have provided comprehensive insights that are directly applicable to real-world scenarios. While extending the VDE to 1 cm could potentially offer additional insights, we believe the current scope sufficiently addresses the research questions posed and provides valuable contributions to the field.

Page 8 Line 163-165: At which injection pressure did the authors apply the GLP-1 analogue?
RESPONSE: Thank you for your inquiry regarding the specific conditions under which the GLP-1 analogue was jetted. The observed peak-pressure was 14±1.5 Bar (n=48). The $MiDe_{RadAuto}$ overall jetting characteristics are plotted in Extended data fig. 8b and are described in the description of the $MiDe_{RadAuto}$ in Supplementary information – Methods Section 6. We have added the pressure value to the legend of Fig. 4c for better readability. We trust that this clarification enhances the comprehensibility of our methods and allows for a more thorough understanding of the experimental setup.

Page 17 Fig.4 a,b,c, and e: Data points at/after the 8h time-point are not visible or partially visible. Please correct the graph image accordingly.
RESPONSE: Thank you for catching this important detail. We have made the necessary corrections to the graph to ensure that all data points are clearly visible.

Page 46-48 825-845: Given the intricate nature of this sophisticated system, how would you judge its overall biodegradability and sustainability? Furthermore, how would you evaluate its scalability for production on a broader scale to cater to a larger patient population?
RESPONSE: Thank you for your touching upon the development aspects of manufacturability, environmental sustainability and scalability. In the rapidly evolving landscape of biodegradable materials, various options are being explored. For instance, there is a wide array of plastics marketed, such as polylactic acid (PLA) and polyhydroxyalkanoates (PHA), although they may not yet possess the necessary mechanical properties. Additionally, biodegradable metals, including magnesium alloys, are also being investigated for potential use in medical devices.
Comparing the complexity of the components in the presented system to that of commercial single-dose pen injectors reveals that the former does not exceed the observed intricacy in existing devices. Moreover, the manufacturing tolerances are within feasible ranges, thereby avoiding the need for micro-scale precision. Notably, the most significant tolerance we have identified pertains to the nozzle diameter. However, it is worth mentioning that there are already marketed needle-free injection devices for subcutaneous administration, applying highly scalable injection moulding processes for manufacturing, proving it's scalability.
In light of these considerations, while the concerns you have raised regarding biodegradability, sustainability, and scalability are indeed valid and essential for commercialization efforts, we have not encountered any clear showstoppers at present. We recognize the need to thoroughly consider and address these concerns as we move forward with our commercialization endeavours.
Based on your comment we have now integrated this aspect in the Discussion section of the manuscript (page 12, lines 315-318). Thank you for your valuable insights, and we will ensure that these aspects are carefully considered in the next stages of development and commercialization.

Page 13 Fig.1f&g: The visibility of the letters f&g is significantly compromised by the whitish background. This issue could be addressed by changing it to black.
RESPONSE: Thank you for catching another important detail. We have made the necessary corrections to Fig. 1f,g to ensure that all captions are clearly visible.

Page 13 Fig.1f: Incorporating a time and length scale would enhance the comprehension of the propulsion/expulsion phenomenon.
RESPONSE: Thank you for your suggestion. We have incorporated your comment to Fig. 1f to ensure that all data points are clearly visible.

Reviewer Reports on the First Revision:

Referees' comments:

Referee #1:
Remarks to the Author:
Thank you for addressing my comments

Referee #2:
Remarks to the Author:
The authors have addressed all the concerns of this Reviewer. The revised manuscript has been significantly improved and is ready for publication.

Referee #3:
Remarks to the Author:
The authors have adequately responded to all my questions and revised their manuskript accordingly. For me, it would be now perfectly acceptable for publication. My compliments toi this nice piece of work!

Referee #4:
Remarks to the Author:
Thank you for asking me to review this interesting paper in which Arrick et al outline the engineering principles and a proof-of-concept for delivery of peptide or high MW-medicines to the GI tract using a novel needle-less jet technology. In principle this improves the bioavailability of such medications which would usually be delivered using subcutaneous injections or similar technologies, up to 90%, although I note these experiments were not performed using the MiDeAxAuto device which would be in principle the device that would be swallowed by patients for delivery of such drugs. I will not comment on the engineering aspects but commenting from my perspective as a clinician who looks after metabolic diseases.

Firstly, I note that subcutaneous injections are a mature technology and allow for precise dosing of the desired medication. It is not clear to me that the MiDe devices might be as precise in delivery – this point is critical, especially for drugs such as insulin where too much or too little, too early or too late can lead to hypo or hyperglycaemia.

Secondly, I note that certain classes of drugs such as the GLP-1 analogues are moving towards 'small molecule' orally bioavailable molecules. These drugs will be far more cheaply available than a peptidic GLP-1 analogue delivered using a MiDe device and will be formulated into tablets using very well understood pharmaceutical manufacturing methods, thus undermining the case of using these

devices. Of course, this will differ from class to class: for insulin, for example, no manufacturer has as yet made a commercially viable non-injection formulation, and for monoclonal antibodies, these devices may be viable.

Thirdly, in practice, there may be issues with patient acceptability and practicability in real life. Relatively large tablets (for example metformin SR tablets which are roughly 8mm long, 4 mm wide and deep) are already at the upper limit of acceptability for some patients who dislike taking large tablets. Capsule endoscopes are probably about the same dimensions as the MiDeAxAuto device but are only really occasionally used and not regularly taken. Furthermore, I assume that these devices would need to be consumed in the fasting state with a delay before eating to allow for consistent passage into the correct area of the small bowel - this may not work for drugs such as rapid-acting insulin, which would need to be administered in the context of eating.

Fourth, there will be many circumstances in which GI anatomy or functional variations may change the delivery characteristics of the devices and make the devices unsuitable. For example, strictures may prevent passage of devices and possibly lead to bowel obstruction - this is already a practical issue for capsule endoscopes. As another example, patients with GI surgery (eg Roux-en-Y gastric bypass or sleeve gastrectomy) may not be suitable for MiDe devices. Gastroparesis is also a common issue which may confound delivery of MiDe devices. I would also note that a pH-sensitive capsule system may be confounded by situations in which the pH of the stomach may be less acidic than expected, such as with proton pump inhibition or with achlohydria.

Lastly, this may be an overly technological solution for a problem that in practice is not a big one, as I find in clinical practice subcutaneous injections are reasonably acceptable especially if dosing frequencies such as weekly injections are required. Perhaps we should be moving in the direction of simpler solutions – for example, the small molecule GLP-1 analogues which can be delivered at cheaper prices to a wider global audience.

This is not to take away from the very nice work that the authors have made, and I applaud their persistence in looking at alternative methods to deliver medications. The MiDe devices do have one advantage over conventional subcutaneous devices in that they may entail less plastic waste and hopefully will be easier to manufacture. I accept that for certain applications (e.g. drugs that really cannot be delivered subcutaneously, or that require specific targeting to the portal circulation) that the MiDe devices might be useful, but this is more likely to be a niche indication.

Author Rebuttals to First Revision:

Referee 4 Comments

Thank you for asking me to review this interesting paper in which Arrick et al outline the engineering principles and a proof-of-concept for delivery of peptide or high MW-medicines to the GI tract using a novel needle-less jet technology. In principle this improves the bioavailability of such medications which would usually be delivered using subcutaneous injections or similar technologies, up to 90%, although I note these experiments were not performed using the MiDeAxAuto device which would be in principle the device that would be swallowed by patients for delivery of such drugs. I will not comment on the engineering aspects but commenting from my perspective as a clinician who looks after metabolic diseases.

RESPONSE: Thank you very much for your assessment and review of our manuscript, including the positive feedback and the constructive suggestions for improvement. We employed the endoscopic-supported devices ($MiDe_{AxEndo}$ and $MiDe_{RadEndo}$) to investigate the feasibility of our approach under controlled conditions. Their evaluation and controlled application in the GI tract allowed us to precisely control and monitor the delivery process ensuring accurate assessment of the bioavailability potential of jetting in the GI tract. Moreover, we recognize the potential of endoscopic-mediated jet application as a potential expansion of endoscopic therapeutic interventions. The bioavailabilities achieved with different devices are clearly tabulated in Fig. 4 for transparency. We understand the importance of evaluating and further optimizing the bioavailability achieved with autonomous devices like the MiDeAxAuto and MiDeRadAuto, which may be employed for patient self-dosing. We have now acknowledged this need for further engineering improvements in our Discussion section.

Firstly, I note that subcutaneous injections are a mature technology and allow for precise dosing of the desired medication. It is not clear to me that the MiDe devices might be as precise in delivery – this point is critical, especially for drugs such as insulin where too much or too little, too early or too late can lead to hypo or hyperglycaemia.

RESPONSE: Thank you for this important comment. MiDe technology enables endoscopic or autonomous/oral delivery of a broad set of macromolecule therapeutics to the GI tract, with bioavailabilities approaching those of parenteral administration. We recognize that drugs with narrow therapeutic windows such as short/rapid-acting insulin may not be amenable to the oral route of administration, which is recognized at baseline to have broad variability in its absorption characteristics. Gastric delivery may help mitigate this, given the short/rapid time frame, but this would have to be informed by future clinical studies. We have now added these considerations to the Discussion section.

Secondly, I note that certain classes of drugs such as the GLP-1 analogues are moving towards 'small molecule' orally bioavailable molecules. These drugs will be far more cheaply available than a peptidic GLP-1 analogue delivered using a MiDe device and will be formulated into tablets using very well understood pharmaceutical manufacturing methods, thus undermining the case of using these devices. Of course, this will differ from class to class: for insulin, for example, no manufacturer has as yet made a commercially viable non-injection formulation, and for monoclonal antibodies, these devices may be viable.

RESPONSE: Thank you for this comment. We would like to reiterate that our goal is to introduce a new paradigm for the administration of therapeutics via the GI tract. Our MiDe technology enables the oral delivery of a broad set of macromolecules, including proteins, nucleic acids (e.g. siRNA), monoclonal antibodies, with bioavailabilities exceeding 10%. To demonstrate this technological advance, we selected the GLP-1 analogue, semaglutide, as a model therapeutic, given our familiarity with its formulation and bioanalysis. This point is now more thoroughly articulated in the Summary and Main sections. We recognize that significant efforts to develop small-molecule alternatives to peptidic GLP-1RAs exist. However, we believe that MiDe technology could enable the delivery of a broad array of therapeutics currently restricted to parenteral routes. Furthermore, the platform could provide rapid relief to the supply issues facing currently approved oral GLP-1RA therapies in the US by the significant reduction in the required mass of the active pharmaceutical ingredient that is currently required in oral formulations of GLP-1RAs. We have now added these considerations to the Discussion section.

Thirdly, in practice, there may be issues with patient acceptability and practicability in real life. Relatively large tablets (for example metformin SR tablets which are roughly 8mm long, 4 mm wide and deep) are already at the upper limit of acceptability for some patients who dislike taking large tablets. Capsule endoscopes are probably about the same dimensions as the MiDeAxAuto device but are only really occasionally used and not regularly taken.

RESPONSE: Thank you for highlighting this critical point regarding patient preference and acceptability. Considering the dimensions of commonly used solid dosage forms (e.g. OROS), further miniaturization is

indeed a critical engineering consideration. Additionally, it is worth noting recent findings that highlight patient preferences for oral delivery over parenteral routes. A recently published clinical study involving 150 participants, indicated that 91% of patients would switch to orally administered biologics and all were able to swallow the 000 size capsule (https://doi.org/10.2147/PPA.S463354). We have added this information to the first paragraph of the Main section.

Furthermore, I assume that these devices would need to be consumed in the fasting state with a delay before eating to allow for consistent passage into the correct area of the small bowel - this may not work for drugs such as rapid-acting insulin, which would need to be administered in the context of eating.
RESPONSE: Thank you for raising this important point in relation to food intake. These devices administer therapeutics through physical means, like a hypodermic needle. Just as a subject may expose their skin for self-administration, the tissue or site where the injection is to take place should have an un-impeded path. While it is true that bolus insulin would need to be taken before a meal, given the inherent variability (see above), this class of drugs might not be the best fit for the proposed solution. However, we would like to highlight that compared to $MiDe_{RadAuto}$, pH-sensitive devices designed to deliver their payload to the small intestine could potentially mitigate some of the food effect challenges, as the liquid chyme might not impede successful delivery. This aspect, of course, needs to be thoroughly investigated in clinical studies. In the Discussion section, we have now expanded on the considerations around concomitant food intake.

Fourth, there will be many circumstances in which GI anatomy or functional variations may change the delivery characteristics of the devices and make the devices unsuitable. For example, strictures may prevent passage of devices and possibly lead to bowel obstruction - this is already a practical issue for capsule endoscopes. As another example, patients with GI surgery (eg Roux-en-Y gastric bypass or sleeve gastrectomy) may not be suitable for MiDe devices. Gastroparesis is also a common issue which may confound delivery of MiDe devices. I would also note that a pH-sensitive capsule system may be confounded by situations in which the pH of the stomach may be less acidic than expected, such as with proton pump inhibition or with achlohydria.
RESPONSE: Thank you for raising these points. And indeed there are contraindications and clinical considerations that need to be taken into account when one would be considering using these systems. We have now reflected this important set of constraints in Discussion section of the manuscript.

Lastly, this may be an overly technological solution for a problem that in practice is not a big one, as I find in clinical practice subcutaneous injections are reasonably acceptable especially if dosing frequencies such as weekly injections are required. Perhaps we should be moving in the direction of simpler solutions – for example, the small molecule GLP-1 analogues which can be delivered at cheaper prices to a wider global audience.
RESPONSE: Thank you for your comment. While it is true that subcutaneous injections are reasonably acceptable for some patients, especially with less frequent dosing requirements, there are other factors to consider. Fear of injectables is a recognized barrier, leading to non-adherence and late treatment uptake. Moreover, the training and education required for proper injection technique can present a significant access challenge globally. Additionally, injectables also introduce the challenge of managing sharps. It is also important to note that patient preference might be shifting towards non-injectable options. Alternative delivery methods could potentially improve patient compliance and outcomes. In the first paragraph of the Main section, we have now added a recent reference to support patient preference for oral delivery of biologics as also reflected above.

This is not to take away from the very nice work that the authors have made, and I applaud their persistence in looking at alternative methods to deliver medications. The MiDe devices do have one advantage over conventional subcutaneous devices in that they may entail less plastic waste and hopefully will be easier to manufacture. I accept that for certain applications (e.g. drugs that really cannot be delivered subcutaneously, or that require specific targeting to the portal circulation) that the MiDe devices might be useful, but this is more likely to be a niche indication.
RESPONSE: Once again, thank you very much for your assessment and review of our manuscript, including the constructive suggestions for improvement. We hope that you consider our revisions in response to your input appropriate.