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Design of a self-unfolding delivery concept for oral administration of macromolecules

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Abstract

Delivering macromolecular drugs, e.g. peptides, to the systemic circulation by oral administration is challenging due to their degradation in the gastrointestinal tract and low transmucosal permeation. In this study, the concept of an oral delivery device utilizing an elastomeric material is presented with the potential of increasing the absorption of peptides, e.g. insulin. Absorption enhancement in the intestine is proposed as a result of self-unfolding of a polydimethylsiloxane foil upon release from enteric coated capsules. A pH-sensitive polymer coating prevents capsule disintegration until arrival in the small intestine where complete unfolding of the elastomeric foil ensures close contact with the intestinal mucosa. Foils with close-packed hexagonal compartments for optimal drug loading are produced by casting against a deep-etched silicon master. Complete unfolding of the foil upon capsule disintegration is verified *in vitro* and the insulin release profile of the final delivery device confirms insulin protection at gastric pH. *In vivo* performance is evaluated with the outcome of quantifiable plasma insulin concentrations in all rats receiving duodenal administration of the novel delivery device. By taking advantage of elastomeric material properties for drug delivery, this approach might serve as inspiration

44 for further development of commercially viable biocompatible devices for oral delivery of
45 macromolecules.

46 **1. Introduction**

47 Oral administration of macromolecules, e.g. peptides and proteins, has been attempted for
48 decades in order to improve patient compliance [1,2]. Previous initiatives have mainly
49 focused on incorporating protease inhibitors, such as soybean trypsin inhibitor (STI), and
50 permeation enhancers (PEs) in order to decrease enzymatic degradation while increasing
51 absorption from the intestine [1,3]. Co-formulating peptides with such active excipients in
52 tablets or capsules has been the strategy in several preclinical and clinical assessments
53 [2]. However, innovative oral delivery devices have, over the last few years, challenged the
54 conventional concepts of oral systemic absorption by proposing the application of
55 gastrointestinal (GI) injections resulting in >10% oral bioavailability relative to
56 subcutaneous (SC) injection [4–7]. Physical perforation of the outer layers of the
57 absorptive barriers has thereby become an interesting alternative to excipient-induced
58 absorption of macromolecules [8]. While both approaches are interesting platforms for
59 peptide delivery, the risks of repeated administrations of PEs and GI-injections are
60 unknown. Nevertheless, the constant regeneration of the epithelial cells along the GI-tract
61 is a recurring argument for the therapeutic relevance of temporary barrier disruption
62 regardless of being induced by PEs or by physical perforation [5,9,10].

63 The status as food additive, Generally Recognized As Safe (GRAS) or inactive ingredient
64 held by some PEs, e.g. sodium caprate (C₁₀), salcaprozate sodium (SNAC) and sodium
65 dodecyl sulfate (SDS), have rendered them popular choices in industrial formulation
66 strategies [8]. Notably, C₁₀ and SNAC have proven beneficial, and have led to the
67 achievement of therapeutic oral bioavailability of insulin and the glucagon-like peptide-1
68 agonist, semaglutide, respectively. Moreover, the latter formulation has received approval
69 from the U.S. Food & Drug Administration [11,12]. Thereby, PE-based formulations have
70 proven to be commercially viable, yet they still need to compensate with higher peptide
71 doses due to relatively low oral bioavailability compared to the GI-injecting delivery devices
72 [5,7,11,12]. On the other hand, while the GI-injecting devices are innovative from an
73 engineering perspective, their complexity is likely to impede the rate of potential mass
74 production compared to that of conventional tablets and capsules [4,5,7]. A solution to the
75 high doses required in PE-based oral dosage forms has previously been proposed with
76 unidirectional-releasing devices capable of creating local environments of high

77 concentrations of peptide and excipients along the intestinal absorptive barrier [13–16].
78 Such confinement would increase the local effect of active excipients, e.g. PEs and
79 protease inhibitors, while creating a steep peptide concentration gradient across the
80 intestinal mucosa. The proximity between the absorptive barrier and such unidirectional-
81 releasing devices has proven of outmost importance *in vitro* with a 50% decrease in insulin
82 absorption for every distance increase of 130 μm from a Caco-2 cell monolayer mimicking
83 the intestinal absorptive barrier [16].

84 Insulin release in the gastric environment can be prevented by enteric coating, but
85 ensuring optimal unidirectional release in close proximity to the intestinal epithelium has
86 proven challenging to attain *in vivo* [17]. Moreover, it has proven problematic to conduct *in*
87 *vivo* studies of enteric coated capsules in rats, due to long-term gastric retention of such
88 dosage forms [18]. Previously, this issue has been circumvented by pre-administration of
89 the prokinetic agent, metoclopramide, to promote gastric emptying, or by direct intestinal
90 insertion of the dosage form through an incision in the proximal small intestine [13,14,19].
91 Thus, such preclinical *in vivo* studies either alter the normal peristalsis by inclusion of a
92 prokinetic agent or might even possess the risk of absorption at the site of intestinal
93 perforation, thereby causing a false positive result.

94 An alternative approach to preclinical *in vivo* studies of enteric coated capsules in rats was
95 used in the present work, without neither penetrating the GI-tract mucosa nor using
96 prokinetic agents. This *in vivo* approach, which will be discussed in more detail below,
97 contributed to the overall aim of the present study, namely to conceptualize and design an
98 oral device capable of guaranteeing optimal unidirectional drug and excipient release in
99 close proximity to the intestinal mucosa. The design comprises an elastomeric self-
100 unfolding foil with cavities for drug- and excipient loading rolled up in an enteric coated
101 gelatin capsule. The key to successfully achieving unidirectional release in close proximity
102 to the intestinal mucosa thus lies in the elastomeric nature of the material ensuring purely
103 elastic deformation. For the present proof-of-concept study, polydimethylsiloxane (PDMS)
104 was chosen as the foil material due to its elastomeric properties, while the approximately 6
105 kDa peptide hormone, insulin, was chosen as the model macromolecular drug.

106 2. Materials and Methods

107 2.1 Materials

108 Human recombinant insulin, SDS and dibutyl sebacate were acquired from Sigma-Aldrich
109 (St. Louis, MO, USA). STI was bought from Thermo Fisher Scientific (Waltham, MA, USA)
110 and SYLGARD™ 184 silicone elastomer kit from Dow Chemical Company (Midland, MI,
111 USA). Eudragit® L 100 and L100-55 were both obtained from Evonik (Essen, Germany).
112 Midazolam (5 mg mL⁻¹) was procured from Hameln (Gloucester, UK) whereas Hypnorm
113 (fentanyl, 0.315 mg mL⁻¹; fluanisone 10 mg mL⁻¹) was acquired from Skanderborg
114 Pharmacy (Skanderborg, Denmark), and pentobarbital/Euthanimal (400 mg mL⁻¹) from
115 Alfasan (Woerden, Netherlands). All additional chemicals and solvents were at least of
116 analytical grade and obtained from commercial suppliers. Ultrapure water was used
117 throughout the studies purified by an Ultra Clear UV system (Evoqua Water Technologies,
118 Pittsburgh, PA, USA).

119 2.2 Fabrication of hexagonal patterned silicon master

120 For optimal results during the deep anisotropic etch into the silicon (Si) substrate, a SiO₂
121 hard mask was used. A 1 µm thick wet thermal oxide was grown in a horizontal furnace
122 (Tempress, Vaassen, The Netherlands) and masked using the conventional positive
123 photoresist AZ® 5214 E (MicroChemicals, Ulm, Germany). In order to increase resist
124 adhesion, hexamethyldisilazane was deposited as part of the procedure for spin coating the
125 1.5 µm thick resist layer. The UV-exposure was conducted using an MLA100 Tabletop
126 Maskless Aligner (Heidelberg Instruments, Heidelberg, Germany) and a dose of 90 mJ cm⁻²
127 before developing the exposed pattern for 90 s using AZ® 726 MIF (MicroChemicals,
128 Ulm, Germany). Subsequently, the hexagonal pattern was transferred into the oxide layer
129 using an Advanced Oxide Etcher (STS MESC Multiplex ICP, SPTS Technologies,
130 Newport, UK) with C₄F₈ and H₂ as the reactive gasses. The remaining resist mask was
131 stripped using a combination of energetic oxygen plasma in a barrel asher (300 Semi Auto
132 Plasma Processor, PVA TePla, Wettenberg, Germany) and submersion into concentrated
133 H₂SO₄ with (NH₄)₂S₂O₈ salt at 80 °C. The honeycomb trenches were then etched into the
134 Si using an inductively coupled plasma deep reactive ion etching tool (STS Pegasus,
135 SPTS Technologies, Newport, UK). The tool utilizes a Bosch-type process for performing
136 deep anisotropic etching of Si by alternating between Si etching using SF₆ and O₂ and
137 sidewall passivation obtained by deposition of C₄F₈. The substrate temperature was kept

138 at 0 °C throughout the etching process and the substrate was cleaned using oxygen
139 plasma and a mixture of concentrated H₂SO₄ and H₂O₂ (4:1 v/v, commonly referred to as
140 piranha) before stripping the remaining oxide mask in aqueous buffered hydrofluoric acid
141 (12%, v/v) with NH₄F. As the Si master was intended for foil fabrication by means of
142 casting with PDMS, an anti-stick coating was deposited by molecular vapor deposition
143 (MVD 100 Molecular Vapor Deposition System, Applied Microstructures, Orbotech, Yavne,
144 Israel). This effectively created a monolayer of 1H,1H,2H,2H-perflourodecyltrichlorosilane,
145 which decreased the surface energy of the Si master. This in turn promoted demolding of
146 the delicate honeycomb protrusions after the PDMS casting and ensured that elastomer
147 rip-off was prevented, thereby preserving the pristine master for multiple replication cycles.
148 The width and depth of the hexagonal trenches were measured by vertical scanning
149 interferometry using a PLu Neox 3D Optical Profiler (Sensofar Metrology, Terrassa,
150 Spain).

151 *2.3 Fabrication of PDMS foil*

152 The self-unfolding elastomer foils were fabricated by casting with SYLGARD™ 184, which
153 is a two-component product consisting of a PDMS base and a curing agent. The base
154 resin and curing agent were mixed in a 10:1 ratio (w/w) and degassed in a desiccator for
155 30 min prior to use. The mixture was then poured onto the silicon master and degassed in
156 a desiccator once more in order to remove potential air bubbles, which would otherwise
157 compromise the structure replication fidelity. The uncured PDMS replica was then kept in
158 an oven at 37 °C overnight and the cured elastomer foil was subsequently peeled from the
159 Si master. The topography of the foils was characterized using vertical scanning
160 interferometry on a PLu Neox 3D Optical Profiler (Sensofar Metrology, Terrassa, Spain). *In*
161 *vitro* assessment of the self-unfolding properties of the foil was assessed by recording the
162 unfolding of a PDMS foil (7 × 7 mm²) as a result of capsule disintegration in water at 37 °C
163 using a Dino-Lite Premier AM7013MZT digital microscope (AnMo Electronics Corporation,
164 New Taipei City, Taiwan).

165 *2.4 Preparation of the final oral delivery device*

166 PDMS foils were loaded with a powder mixture of insulin, SDS as PE and STI (5:3:2,
167 w/w/w) by tapping the powder on top of the foil and gently scraping off any excess amount.
168 A solution of Eudragit® L 100 (1%, w/v) and dibutyl sebacate (0.1%, w/v) in isopropanol
169 (IPA) was then spray coated on top of the hexagonal openings to seal the powder inside

170 thus enabling subsequent handling of the foils. An ExactaCoat Ultrasonic Spray System
171 (Sono-Tek, Milton, NY, USA) was used for this purpose with an infuse rate of 0.05 mL min⁻¹,
172 path speed of 5 mm s⁻¹ and shaping air pressure of 0.02 mbar. The generator power
173 was set to 2.2 W and an AccuMist nozzle was applied with a sample-to-nozzle distance of
174 50 mm. Immediately after spray coating, the foils were gently rolled between the finger tips
175 and inserted into size 9 gelatin capsules (Torpac, Fairfield, NJ, USA). A flat Si-chip was
176 additionally coated to determine the coating thickness of Eudragit® L 100 by scanning
177 electron microscopy using a Hitachi TM3030 Plus tabletop microscope (Hitachi High-
178 Technologies Europe, Krefeld, Germany) with an accelerating voltage of 15 kV. Nine
179 delivery devices were prepared in total out of which three were used for testing for the
180 uniformity of insulin content, three were used for *in vitro* insulin release studies and three
181 prepared for *in vivo* insulin absorption studies. The gelatin capsules for *in vivo* studies
182 were additionally loaded with a cylindrical power magnet with a diameter and height of 2 ×
183 1 mm, respectively (Magnetz og Magnordic, Hvidovre, Denmark). Enteric coating of the
184 gelatin capsules was carried out by two dip coating cycles, first dipping the capsule body
185 and afterwards the cap in Eudragit® L 100-55 (15%, w/v) and dibutyl sebacate (0.75%,
186 w/v) in IPA giving the final delivery device. Images of the delivery device were captured
187 with a Dino-Lite Premier AM7013MZT digital microscope (AnMo Electronics Corporation,
188 New Taipei City, Taiwan).

189 2.5 *In vitro* release of insulin

190 A test for uniformity of insulin content was initially carried out by fully dissolving the content
191 of three delivery devices in 10 mL of 50 mM phosphate buffer at pH 7 followed by reversed
192 phase high-performance liquid chromatography (HPLC) as described below. *In vitro*
193 release studies were carried out on a 400-DS Apparatus 7 (Agilent Technologies, Santa
194 Clara, CA, USA) equipped with 5 mL sample cells and supplied with a 50 mM citrate buffer
195 at pH 4 and a 50 mM phosphate buffer at pH 7. The oral delivery device was placed in 50-
196 mesh basket sample holder and dissolution carried out under sink conditions at 37 °C for 1
197 hour at pH 4 followed by 5 hours at pH 7 with a dip speed of 15 dips per minute. Automatic
198 sampling of 0.5 mL was timed at 10, 30, 60, 70, 80, 90, 105, 120, 150, 180, 240 and 360
199 min, each replaced with fresh dissolution medium. After sampling at 60 min, the total
200 volume of citrate buffer in the sample cell was fully replaced with phosphate buffer. Insulin
201 quantification was immediately carried out by HPLC on a Dionex Ultimate 3000 system
202 (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Kinetex XB-C18 column
203 (100 × 4.6 mm, 5 µm, 100 Å; Phenomenex, Torrance, CA, USA). The following gradient of

mobile phase A: 0.1% (v/v) trifluoroacetic acid (TFA) in water and B: 0.1% (v/v) TFA in acetonitrile was applied with a flow rate of 0.5 mL min⁻¹: 0 – 6.0 min A:B (77:23 to 50:50, v/v), 6.0 – 6.5 min A:B (50:50 to 77:23, v/v) and 6.5 – 8.0 min A:B (77:23, v/v). Insulin was quantified as the area under the curve of the UV-absorbance peak at 276 nm against a standard curve from 10 - 1000 µg mL⁻¹ with an injection volume of 20 µL and column temperature of 22 °C.

2.6 *In vivo* absorption of insulin

The animal experiments were carried out in accordance with the Danish law on animal experiments as approved by the Danish Animal Experiments Inspectorate in concordance with the EU directive 2010/63/EU under license no. 2016-15-0201-00892. In total, 11 male Sprague Dawley rats (Janvier Labs, Le Genest-Saint-Isle, France) weighing 255 – 310 g were divided into three groups and housed with reversed day/night rhythm (12/12 h). The rats were fasted with *ad libitum* access to water for 12 - 14 h before initiating the study. Bolus anesthesia of fentanyl (236 µg kg⁻¹), fluanisone (7.5 mg kg⁻¹) and midazolam (3.75 mg kg⁻¹) was given as SC injections with repeated SC injections every 30 min of 1/3 of the bolus dose together with 0.4 mL saline. The abdominal cavity was opened to expose the gastrointestinal tract prior to dosing of all 11 rats. Two groups, each of three rats, were administered SC injections of insulin (1 IU kg⁻¹) or saline as positive and negative control, respectively. Five rats were administered the novel delivery device directly to the stomach by the use of an oral gavage dosing tube. Gastric emptying was then facilitated by dragging the delivery device into the duodenum about 3 cm from the pylorus with an external magnet (t = 0 min). Blood samples of 200 µL were taken from the tail vein into Microvette® 200 K3E tubes (Sarstedt, Nümbrecht, Germany) at 10, 20, 30, 45, 60, 90, 120 and 180 min followed by isolation of the plasma by centrifugation at 9,300 × g at 4 °C using a Microcentrifuge 5415 R (Eppendorf, Hamburg, Germany). Plasma samples were stored at -20 °C until insulin quantification was carried out by an enzyme-linked immunosorbent assay (ELISA) as described by the manufacturer (Mercodia, Uppsala, Sweden). The rats were euthanized by intracardiac injections of pentobarbital (100 mg kg⁻¹) after the final blood sampling. Additionally, diluted *in vitro* release samples were analyzed by ELISA in order to confirm preservation of the antibody-binding moieties of insulin upon release from the prepared delivery device.

2.7 *Data treatment*

236 All data were treated using Microsoft Excel 2010 (Redmond, WA, USA) and GraphPad
237 Prism version 8.3.0 (San Diego, CA, USA) and expressed as mean $\pm$ standard deviation
238 (SD) unless stated otherwise.

239 **3. Results and discussion**

240 *3.1 Preparation and in vitro assessment of the foil-based delivery device*

241 An overview of the preparation steps of the drug loaded elastomeric foil is shown in Fig.
242 1A, and the principle of its proposed absorption enhancing mechanism illustrated in Fig.
243 1B.

245 Fig. 1. Illustrations of the preparation steps and the principle of the delivery device (A) Fabrication
246 and preparation steps of drug-loaded polydimethylsiloxane (PDMS) foil with scanning electron
247 microscopy (SEM) images, scale bars: 400 μ m (B) Principle of the oral delivery device: Intestinal
248 capsule disintegration is followed by foil unfolding and unidirectional drug release in close proximity
249 to the absorptive barrier (the gastrointestinal tract schematic was created with Biorender.com) - (2-
250 column fitting image).

251 An early 1st generation design of the cavities for drug- and excipient loading in the PDMS
252 foil was based on previously published cylindrical devices [20,21]. This 1st generation foil

253 designed with cylindrical cavities was used for initial *in vitro* assessment of the self-
254 unfolding properties of the PDMS foil (Fig. 2, Video S1).

256 Fig. 2. Real-time disintegration of a size 9 gelatin capsule in water at 37 °C following unfolding of a
257 PDMS foil with cylindrical cavities, timer shown as MM:SS - (1.5-column fitting image).

258 The confined shape of the foil in the gelatin capsule proved completely reversible with the
259 foil fully restoring its original flat shape upon capsule disintegration. Inside the tubular
260 confinement of the rat duodenum, measuring about 2.5 – 3 mm in diameter, such
261 unfolding would thus result in close proximity between the drug loaded cavities of the foil
262 and the intestinal mucosa [22]. To further enhance the chances of oral insulin absorption,
263 the Si master with a close-packed hexagonal tiling was deep-etched and used for
264 fabrication of the 2nd generation foil (Fig. 1A). The width and depth of the hexagonal
265 trenches of the Si master were 46 ± 2.2 and 127 ± 0.8 μm (mean $\pm$ SD), $n = 5$),
266 respectively (Fig. S1A). Subsequent PDMS casting resulted in thin foils with a well-
267 defined surface topography composed of protrusions arranged in a honeycomb pattern
268 with an average height of 125 ± 0.6 μm (mean $\pm$ SD, $n = 5$), (Fig. S1B). The hexagonal
269 design of the 2nd generation foil resulted in a functional area for drug- and excipient loading
270 of 78% compared to only 21% for the 1st generation foil with cylindrical cavities. The
271 elastomeric PDMS foil with hexagonal cavities was cut into pieces of 7×7 mm^2 , and
272 loaded with a powder mixture consisting of insulin, SDS and STI. A covering layer of

273 Eudragit® L 100, soluble in intestinal fluids with pH > 6, was applied by spray coating to
 274 seal the powder mixture inside the cavities. The covering layer, measuring approximately
 275 19 µm (Fig. S2), thus ensured that no loss of loaded powder occurred when rolling up and
 276 inserting the foil into size 9 gelatin capsules. Finally, dip coating of the gelatin capsules
 277 was performed using Eudragit® L 100-55 to allow for rapid capsule disintegration when
 278 reaching a pH value above 5.5 in the rat small intestine. The uniformity of insulin content in
 279 the delivery devices was quantified as 606 ± 65 µg corresponding to 17.5 ± 1.9
 280 international units (IU), when loading a powder mixture of insulin, SDS and STI (5:3:2
 281 w/w/w). Insulin release from the final delivery device was investigated *in vitro* in media
 282 simulating the pH in rat gastric and small intestinal environments to confirm sufficient
 283 capsule coating (Fig. 3). The insulin profile confirmed protection even at a relatively high
 284 gastric pH of 4, while the exchange of medium to pH 7 initiated the release of insulin,
 285 which lasted about 2 – 3 h.

286

287 Fig. 3. *In vitro* release of insulin from the final delivery device at pH 4 followed by pH 7 under sink
 288 conditions, mean $\pm$ standard deviation ($n = 3$) - (1-column fitting image).

289 3.2 *In vivo* assessment

290 None of the preparation steps towards the final oral delivery device included processes
 291 that were likely to compromise the physical stability of insulin, e.g. heat, physical stress,
 292 solvents. This was supported by ELISA of diluted *in vitro* release samples, which
 293 confirmed the preservation of the antibody-binding moieties of insulin upon release from
 294 the dosage form. An alternative protocol to standard oral gavage was designed for *in vivo*
 295 evaluation of the delivery device, as studies have shown that enteric coated size 9

capsules may not exit the stomach upon oral administration to rats [18]. The procedure was rendered possible by including a cylindrical magnet in the capsule together with the prepared folded foil (Fig. 4A-B).

Fig. 4. *In vivo* setup for assessment of the delivery device prototype (A) The delivery device comprised of a Eudragit® L 100-55 coated size 9 gelatin capsule loaded with a magnet (arrow) and a Eudragit® L 100 coated PDMS foil loaded with insulin, sodium dodecyl sulfate and soybean trypsin inhibitor, scale bar: 2 mm (B) Cross-sectional view of the capsule showing the coiled PDMS foil inside the enteric coated size 9 gelatin capsule, scale bar: 1 mm (C) Illustration of the *in vivo* setup in two steps: 1. oral gavage of the delivery device to the stomach, 2. gastric emptying of the delivery device by the use of an external magnet (schematic created with Biorender.com) - (1.5-column fitting image).

The delivery device was administered by oral gavage to the stomach of anaesthetized rats followed by duodenal positioning, approximately 3 cm from the stomach, which was enabled by external magnetic maneuvering of the delivery device through the pylorus (Fig. 4C). Blood samples were taken over 3 h after which fully unfolded empty PDMS foils were retrieved from the small intestines (20 – 30 cm from pylorus) with no residual remains of the gelatin capsules (Fig. 5A). Changes in blood glucose levels were not used for evaluating insulin absorption, as previous data have shown significant blood glucose

lowering effects by anesthesia [23]. Instead, insulin absorption was assessed by plasma human insulin quantification by ELISA, as shown in Fig. 5B.

Fig. 5. *In vivo* performance of the delivery device prototype (A) PDMS foil retrieved after the *in vivo* study and placed next to a size 9 gelatin capsule for comparison, scale bar: 2 mm (B) Plasma insulin concentrations in rats as micro international units (IU) per mL after duodenal insertion of the oral delivery (Del.) device compared to subcutaneous injections (SC inj.) of either insulin or saline shown as mean + standard error of the mean ($n = 3-5$) - (2-column fitting image).

Insulin was present in quantifiable concentrations in the plasma of all five rats receiving the foil-based delivery device, although in relatively low concentrations compared to the SC insulin injections. Additionally, no traces of insulin were detected in any of the negative control plasma samples from rats receiving SC saline injections. The relative oral bioavailability (F_{Rel}) of the delivery device compared to the SC insulin injection was found to be $0.12 \pm 0.07\%$ (mean $\pm$ standard error) based on the respective doses and the area under the curve (AUC) of the plasma insulin profiles, calculated by the following formula.

$$F_{Rel} = 100 \cdot \frac{AUC_{oral\ administration} \cdot 1\ IU\ kg^{-1}}{AUC_{SC\ injection} \cdot 63\ IU\ kg^{-1}}$$

Although the relative bioavailability achieved might appear low, oral formulations of the antidiuretic hormone, desmopressin, have previously shown to be both therapeutically and commercially viable with a mean relative bioavailability of 0.1% compared to SC injection [24]. Moreover, commonly investigated peptides for oral delivery, e.g. desmopressin, octreotide and leuprolide, might comprise a smaller absorption challenge compared to insulin due to its larger size (51 amino acids), two-chain structure and higher susceptibility to proteolysis [1,12,25]. However, whether higher bioavailability of such simpler peptides could be achieved by the delivery device remains uncertain at this stage. Other oral delivery strategies have previously resulted in a markedly higher oral insulin bioavailability of $>10\%$, with the highest numbers being based on physical perforation of the mucosa by

GI-injecting delivery devices [4-7]. Another study based on local release of thiolated polycarbophil as PE together with insulin from mucoadhesive patches has previously shown a relative oral bioavailability of 2.2% [26], suggesting that greater absorption by the present unfolding foil might be achievable by incorporating alternative PEs or by improvement of the design of the device itself. The present oral delivery principle could therefore be of general interest to both material scientists and also researchers in the field of oral delivery of peptides and even some small molecule drugs with low oral bioavailability. Class III drug compounds in the Biopharmaceutics Classification System are defined as having high solubility, but low permeability and could possibly benefit from the delivery principle of the foils by creating a local environment of high drug concentration and thus a steep gradient across the intestinal absorptive barrier [27]. However, since the current results are based on an early prototype of utilizing self-unfolding foils, further in-depth studies are needed to investigate the true potential of the delivery concept. Moreover, while the elastomer, PDMS, proved advantageous for achieving self-unfolding properties of the foil, the material possesses the notable disadvantage of not being biodegradable. While PDMS is already used for medical prosthetics and plastic surgery and generally considered nontoxic [28], its lack of disintegration represents a possible risk of accumulation in the GI-tract upon repeated oral administration. Hence biodegradability would be a considerable advantage for proceeding further from preclinical studies, yet a challenge lies in the creation of a biodegradable elastomeric foil with similar properties. Several approaches towards such materials have previously been investigated for biomedical applications based on alginate, poly(glycerol-sebacate) and even combinations of PDMS and starch [28–31]. For the purpose of oral peptide delivery, it might furthermore be of specific interest to investigate different cavity structures for drug loading and the unfolding forces of different elastomers. The interplay between those aspects might aid perforation through the intestinal mucus thus further decreasing the distance between the point of peptide- and excipient release and the absorptive barrier.

4. Conclusion

A new oral delivery concept comprising an elastomeric PDMS foil in an enteric coated gelatin capsule was designed and tested both *in vitro* and *in vivo*. The *in vitro* studies confirmed protection at gastric pH and fully unfolding properties of the PDMS foil upon capsule disintegration. A new *in vivo* assessment for enteric coated capsules was applied to ensure gastric emptying of the designed delivery device, which showed promising properties for oral delivery of macromolecules as all rats had quantifiable insulin plasma

375 concentrations despite insulin being one of the more challenging peptides to deliver orally.
376 In general, drug compounds with low oral bioavailability, due to low permeation and/or
377 stability, could benefit from confinement in and subsequent release from the foil [27,32].
378 Excipient-driven absorption mechanisms of macromolecules might furthermore gain
379 increased efficiency from co-localization, leading to a reduced requirement of drug. Thus,
380 the concept might have the potential to serve as a platform for a range of drug
381 compounds, yet further studies is needed to fully unravel its potential.

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386 National Centre for Nano Fabrication and Characterization at the Technical University of
387 Denmark. The graphical abstract was created with Biorender.com.

388 **Declaration of interest**

389 JRJ, LHET, AB, TR and AM have filed a patent application to the European Patent Office
390 on the design of the delivery device.

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