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**Nielsen, LH, Nagstrup, J, Gordon, S, Keller, SS, Østergaard, J, Rades, T, Müllertz, A and Boisen, A**

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### Article

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1 **pH-triggered drug release from biodegradable microwells for oral drug delivery**

2

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16

17

18 **Keywords:** Biodegradable polymer, oral drug delivery, micro delivery systems, furosemide,  $\mu$ -Diss profiler, UV  
19 imaging.

20

21 **Abstract**

22 Microwells fabricated from poly-L-lactic acid (PLLA) were evaluated for their application as an oral drug delivery  
23 system using the amorphous sodium salt of furosemide (ASSF) as a model drug. Hot embossing of PLLA resulted in  
24 fabrication of microwells with an inner diameter of 240  $\mu\text{m}$  and a height of 100  $\mu\text{m}$ . The microwells were filled with  
25 ASSF using a modified screen printing technique, followed by coating of the microwell cavities with a gastro-resistant  
26 lid of Eudragit<sup>®</sup> L100. The release behavior of ASSF from the coated microwells was investigated using a  $\mu$ -Diss  
27 profiler and a UV imaging system, and under conditions simulating the changing environment of the gastrointestinal  
28 tract. Biorelevant gastric medium (pH 1.6) was employed, after which a change to biorelevant intestinal release medium  
29 (pH 6.5) was carried out. Both  $\mu$ -Diss profiler and UV imaging release experiments showed that sealing of microwell  
30 cavities with an Eudragit<sup>®</sup> layer prevented drug release in biorelevant gastric medium. An immediate release of the  
31 ASSF from coated microwells was observed in the intestinal medium. This pH-triggered release behavior demonstrates  
32 the future potential of PLLA microwells as a site-specific oral drug delivery system.

## 33 **1 Introduction**

34 The oral route is widely considered as the preferred administration method for drug delivery, due to its non-invasive  
35 nature and the possibility for self-administration, which together provide a high patient compliance as well as an  
36 improved safety compared to other administration routes (Balimane et al. 2000; Perioli et al. 2012). However, an  
37 increasing number of new drug compounds exhibit poor aqueous solubility, a slow rate of dissolution and/or poor  
38 intestinal permeability, making effective oral drug delivery increasingly challenging (Bergstrom et al. 2007; Newman  
39 et al. 2008). Innovative drug delivery systems have the potential to facilitate an improvement in oral bioavailability  
40 compared to current drug formulations. Novel approaches such as mucoadhesive gastrointestinal (GI) patches and micro  
41 fabricated devices have been suggested as oral delivery systems for drug compounds (Chirra and Desai 2012; Colombo  
42 et al. 2009; Eaimtrakarn et al. 2001). In particular, microfabricated wells have been proposed as promising oral drug  
43 delivery systems (Ahmed et al. 2002; Ainslie et al. 2009; Tao et al. 2003). These microwells are small structures  
44 consisting of a walled reservoir (into which drug can be incorporated) extending from a flat base. The size and shape of  
45 these microwells can easily be controlled to maximize the contact area with absorptive tissues, such as the intestinal  
46 membrane, providing optimal conditions for drug absorption. Furthermore, in comparison to micro- and nanoparticles,  
47 only one side of microwells is exposed to the external environment. This allows for unidirectional drug release, and also  
48 for protection of the drug in harsh environments, such as that occurring in the stomach (Ahmed et al. 2002; Ainslie et al.  
49 2009; Eaimtrakarn et al. 2001; Tao et al. 2003). Microwells have previously been fabricated using various materials,  
50 including silicon (Ahmed et al. 2002), poly(methyl methacrylate) (Chirra and Desai 2012; Tao and Desai 2005) and SU-  
51 8 (Tao and Desai 2007). The authors have previously presented processes for fabrication of microstructured wells using  
52 the polymers polycaprolactone (PCL) and poly-L-lactic acid (PLLA) (Nagstrup et al. 2011). These materials are  
53 advantageous as they are already used in other medical devices approved by the US Food and Drug Administration.

54  
55 The increasing level of difficulty associated with oral delivery of new as well as established drug candidates is  
56 exemplified by furosemide, a Biopharmaceutics Classification System class IV compound with both a poor aqueous  
57 solubility and a low intestinal permeability. Furosemide is a loop-diuretic and is mainly used for oral treatment of  
58 hypertension and oedema (Matsuda et al. 1990). The employment of an amorphous sodium salt of furosemide (ASSF)  
59 has been previously shown to significantly improve aqueous solubility and dissolution rate in comparison to furosemide  
60 in a crystalline acid form (Nielsen et al. 2013a). However, while the employment of ASSF may aid in countering  
61 solubility and dissolution-related issues, the poor intestinal absorption of furosemide is further complicated by a  
62 tendency to undergo site-specific absorption partly in the stomach, but especially in the upper part of the small intestine,  
63 leading to a considerable inter- and intra-individual variation in oral drug bioavailability (20-60%) (Iannuccelli et al.  
64 2000). There is therefore, a further need to improve furosemide absorption and specifically reduce the variation in  
65 bioavailability, a task which could be accomplished through the use of advanced drug delivery systems, such as  
66 microwells.

67  
68 Typically, drug is filled into microwells using techniques such as microspotting and injection, which allow for loading  
69 of nanoliter quantities of solution into the reservoirs (Ahmed et al. 2002). Following filling, particularly when  
70 consideration of oral delivery, it may be desirable to seal the open side of the filled microwells with a lid in order to  
71 allow for an even greater degree of drug protection during transit prior to arrival at the site of absorption, and also to  
72 provide a level of control over the rate and/or location at which the drug is released. In this respect, Eudragit® polymers  
73 have been successfully employed in many studies as biocompatible coatings for oral dosage forms. Eudragit® polymers  
74 can be utilized to facilitate a slower drug release, which may lead to an enhancement of drug absorption and a  
75 prolonged drug effect (Pandey et al. 2013). They may also be used in order to facilitate controlled drug release in  
76 selected areas of the GI tract (Luppi et al. 2009; Zhu et al. 2011). Eudragit® L100 in particular is a pH-sensitive anionic  
77 copolymer based on methacrylic acid and methyl methacrylate, which dissolves at pH levels higher than 6. Coating of  
78 dosage forms with Eudragit® L100 has been shown to be a very useful strategy for protecting orally-delivered drugs  
79 from the acidic environment of the stomach. Subsequent, dissolution of Eudragit® L100 upon encountering the higher  
80 pH of the small intestine then allows for drug exposure and absorption (Pandey et al. 2013).

81

82 The studies presented to date in the literature demonstrate initial investigations in the employment of microwells as drug  
83 delivery systems (Ahmed et al. 2002; Ainslie et al. 2009; Tao et al. 2003). The current work therefore aims to contribute  
84 to the body of work concerned with optimization of microfabricated devices for oral drug delivery, by first investigating  
85 their potential to be loaded with ASSF as a model drug, and furthermore by determining their ability to protect and  
86 deliver ASSF under conditions approximating those found in the GI tract. To the best of the authors' knowledge, this is  
87 the first time micro-fabricated drug delivery devices have been combined with biopolymers and pH-dependent enteric  
88 coatings. The biodegradable microwells fabricated from PLLA were filled with ASSF using an innovative stencil-based  
89 method depositing the powder drug into the microwells. Filled microwells were then spray coated with a lid of  
90 Eudragit® L100. The release of ASSF from coated microwells was investigated in gastric and intestinal biorelevant  
91 media using a  $\mu$ -Diss profiler and a UV-imaging system, in order to investigate the ability of the Eudragit® coating to  
92 facilitate drug protection and controlled release and to determine the suitability of the microwells as oral delivery  
93 systems.

94

## 95 **2 Materials and Methods**

### 96 2.1 Materials

97 PLLA granulate 2002D was obtained from Nature Works LLC (Blair, USA). Furosemide (>98% purity) and  
98 taurocholic acid sodium salt hydrate (sodium taurocholate) were purchased from Sigma-Aldrich (St. Louis, MO, USA).  
99 Eudragit® L100 was obtained from Evonik Industries (Darmstadt, Germany). Phosphatidylcholine (Lipoid S PC, purity  
100  $\geq$  98% phosphatidylcholine) was obtained from Lipoid AG (Ludwigshafen, Germany). Sodium azide, sodium chloride  
101 and potassium dihydrogen phosphate were acquired from Merck (Darmstadt, Germany). Ultra-purified water was  
102 obtained from an SG Ultra Clear water system (SG Water USA, LLC, Nashua, NH, USA) and was freshly produced in  
103 all cases. All other chemicals used were of analytical grade.

104

### 105 2.2 Methods

#### 106 2.2.1 Fabrication of PLLA microwells by hot embossing

107 The microwells were prepared by hot embossing in PLLA films in a similar manner to shown previously (Nagstrup et  
108 al. 2011). Briefly, a PLLA solution (Nature Works LLC, 2002D, 25 wt% in dichloromethane) was first manually  
109 dispensed onto a 4-inch silicon wafer. Using a spin coating process, the wafer was accelerated to a final spin speed of  
110 500 rpm which was maintained for 50 s in order to obtain a uniform PLLA film. Produced PLLA films were then  
111 degassed for 2 h and baked at 220°C for 1 h. The film thickness was measured using a contact profilometer (Dektak8,  
112 Veeco, Mannheim, Germany) and the films with a thickness of 100-110  $\mu$ m were further used for fabrication of the  
113 PLLA microwells. For this purpose, a nickel stamp was prepared by electroplating. The stamp and the PLLA coated  
114 wafers were brought in contact in a hot embossing system (EV Group 520, St. Florian am Inn, Austria) and heated to  
115 120°C with a temperature ramping of 10°C/min. A pressure of 1.9 MPa was applied to emboss the stamp into the  
116 polymer. After 1 h the assembly was cooled down to room temperature, the pressure was released and the stamp was  
117 removed from the polymer. Following hot embossing, the wafers containing PLLA microwells were cut into chips of  
118 12.8 x 12.8 mm<sup>2</sup>.

119

#### 120 2.2.2 Preparation and manufacturing of ASSF

121 Amorphous sodium salt of furosemide (ASSF) was prepared as described earlier (Nielsen et al. 2013a). Briefly, purified  
122 water and ethanol (96%) were mixed in a ratio of 10:1 v/v, and crystalline furosemide acid was added at a concentration  
123 of 0.4 w/v%, together with 5 M NaOH in a molar ratio of 1:1 with furosemide. The resulting solution was spray dried in  
124 a Büchi Mini Spray-Dryer B-290 (Büchi Labortechnik AG, Flawil, Switzerland). Following completion of the process,  
125 the spray dried ASSF was collected and stored in glass vials protected from light. X-ray powder diffraction (XRPD)  
126 analysis was performed after spray drying to confirm formation of ASSF.

127

#### 128 2.2.3 Filling of microwells with ASSF

129 A simple version of a screen printing technique was applied in order to fill the PLLA microwells with ASSF, as shown  
130 in Fig 1. A stencil mask was first fabricated from transparent foil by laser machining using a CO<sub>2</sub> laser (48-5S Duo  
131 Lase, Synrad Inc., USA). The stencil was designed to exhibit holes in a position and of a diameter matching the

132 fabricated microwells with a high level of precision (variation in position/diameter in the low  $\mu\text{m}$  range). The stencil  
133 was aligned with the microwells using an optical microscope (Fig 1, step 1) and brought in close contact. ASSF powder  
134 was then pressed through the stencil (Fig 1, steps 2 and 3), and into the microwells. Finally, the stencil was removed,  
135 effectively removing any excess ASSF powder not located within the microwells (Fig 1, step 4).  
136

#### 137 2.2.4 Coating of drug-filled microwells with Eudragit<sup>®</sup> L100

138 A spray coating system (ExactaCoat, Sono Tek, USA) equipped with an ultrasonic nozzle actuated at 120 kHz (Keller  
139 et al. 2013) was used to deposit Eudragit<sup>®</sup> L100 (dissolved to a 1 wt% solution in isopropyl alcohol) on the cavity of the  
140 drug-filled microwells. The generator power was set to 1.5 W, and the polymer solution was pumped through the nozzle  
141 at a flow rate of 100  $\mu\text{L}/\text{min}$ . Nitrogen gas at a pressure of 10 mbar was used to direct the beam of droplets onto the  
142 microwells. The distance between nozzle and substrate was 40 mm, and the beam diameter on the substrate was  
143 approximately 4 mm. The lateral movements of the nozzle were controlled by an x-y stage and the nozzle path was  
144 defined in the equipment software. The nozzle was moved line-by-line at a speed of 25 mm/s, and the coating was  
145 repeated 60 times to obtain a coating thickness in the  $\mu\text{m}$  range. Following the coating procedure, microwells were  
146 analyzed by scanning electron microscopy (SEM) with a Nova 600 NanoSEM (FEI, The Netherlands).  
147

#### 148 2.2.5 Preparation of simulated gastric and intestinal media

149 A biorelevant medium simulating the composition of fasted state gastric fluid was prepared in accordance with Vertzoni  
150 et al. (Vertzoni et al. 2005). The required volume of a stock solution of phosphatidylcholine in chloroform (20  $\mu\text{M}$ ) was  
151 subjected to a steady stream of nitrogen in order to evaporate the solvent and form a lipid film. 80  $\mu\text{M}$  of sodium  
152 taurocholate was then weighed and added to the lipid film, together with 90 mL of 0.02 M HCl solution. A separate  
153 solution containing 80 mL of 0.02 M HCl, 0.1 mg/mL pepsin and 120.7 mOsm/kg sodium chloride was also prepared.  
154 Following overnight stirring, the solutions were mixed, diluted with 700 mL of 0.02 M HCl, adjusted to a pH of 1.6 and  
155 made to volume (1 L) with purified water.  
156

157 A medium simulating the conditions of the fasted intestine was also prepared, as described previously (Nielsen et al.  
158 2013a). The medium utilized for the current studies contained 5 mM of sodium taurocholate as a bile salt and 1.25 mM  
159 of phosphatidylcholine as a phospholipid. The required volume of phosphatidylcholine in chloroform was first exposed  
160 to a flow of nitrogen, resulting in the formation of a lipid film. The volume of a phosphate buffer/sodium azide stock  
161 solution required to give a concentration of 100/3 mM was then added, together with a quantity of sodium chloride  
162 stock solution necessary to achieve a constant osmolarity of 270 mOsm. The medium was stirred overnight at 37°C,  
163 following which the pH was adjusted to 6.5 and the medium was made to volume with purified water.  
164

#### 165 2.2.6 Characterization of ASSF release from Eudragit<sup>®</sup> L100-coated microwells: $\mu$ -Diss profiler

166 The release of ASSF from Eudragit<sup>®</sup>-coated micro wells was investigated in conditions simulating those of the GI tract  
167 using a  $\mu$ -Diss profiler (*p*ION INC, Woburn, MA). Release studies were performed in biorelevant gastric medium for  
168 120 min, followed by a switch to biorelevant intestinal medium for a further 180 min. Before each release experiment, a  
169 standard curve was constructed in each channel of the apparatus in each medium, using *in situ* UV probes with a path  
170 length of 5 mm. In order to prepare standard curves, aliquots of a stock solution of furosemide (in water adjusted to pH  
171 10 with NaOH) were added to medium, and the resulting UV spectrum of the solution was measured. The process of  
172 addition of aliquots followed by spectral measurement was repeated 8 times in order to produce standard curves  
173 covering the entire linear absorbance range. For the release experiments themselves, 12.8 x 12.8 mm<sup>2</sup> chips containing  
174 400 microwells (weighed before and after drug filling in order to allow for accurate determination of the weight of  
175 contained ASSF) were attached to the cylindrical magnetic stirring bar of  $\mu$ -Diss vials. The microwell chips were  
176 covered with 10 mL of biorelevant medium, and spectral collection was initiated. Release experiments in both gastric  
177 and intestinal medium were run at 37°C with a stirring rate of 100 rpm, and recorded spectra were analyzed in the  
178 wavelength range 310-350 nm. Experiments were performed in triplicate and data are presented as mean  $\pm$  standard  
179 deviation (SD).  
180

#### 181 2.2.7 Characterization of ASSF release from Eudragit<sup>®</sup> L100-coated microwells: UV imaging

182 UV imaging experiments were performed in order to gain further information regarding the biorelevant release behavior  
183 of ASSF from Eudragit-coated microwells. Imaging was performed using a Sirius SDI UV imaging system (Sirius  
184 Analytical, East Sussex, UK), equipped with a pulsed xenon lamp as a light source and a quartz flow cell with a light  
185 path of 4 mm (CADISS-3). A 280 nm single wavelength filter was utilized for selection of detection wavelength. The  
186 total area available for imaging was 9 mm x 7 mm (1280 x 1024 pixels). A syringe pump was used for infusion of  
187 biorelevant gastric and intestinal media at a constant flow rate of 0.2 mL/min, and experiments were carried out at  
188 ambient temperature (23-25°C). Images were recorded at a rate of 2.78 frames/s, and were analyzed with Actipix D100  
189 software version 1.4 (Paraytec Ltd.) with a 10 x 1 horizontal pixel binning.

190  
191 In order to perform the release experiments, dark images (lamp off, 10 s duration) and reference images (lamp on, also  
192 10 s total duration) were recorded with the flow cell filled with simulated gastric medium, and with an empty stainless  
193 steel cylinder positioned in the compact holder. Data collection was then initiated and allowed to proceed for 60 s.  
194 Following this period, data recording was paused, the empty stainless steel cylinder was removed, and a cylinder fitted  
195 with a microwell chip section was inserted. A flow of gastric medium was then initiated and simultaneously data  
196 collection was recommenced. After a period of 5 min, data recording was again paused, and the syringe pump was  
197 refitted with a syringe containing biorelevant intestinal medium. Medium flow and data collection were recommenced,  
198 and were continued for a further 15 min. Experiments were performed in triplicate.

### 200 **3 Results and Discussion**

201 The PLLA microwells in this work were fabricated using hot embossing with a nickel stamp. The fabricated microwells  
202 exhibited a uniform surface and proved to be easy to dissociate from the stamp.

203  
204 The fabrication process itself resulted in the formation of microwells set into the PLLA layer on full wafer scale (Fig 2).  
205 The microwells had an outer diameter of 300 µm and an inner diameter of 240 µm. The wall height of produced  
206 microwells was 65 µm, giving a total outer height of 100 µm. These dimensions ensure that the microwells are small  
207 enough to have a good contact with the intestinal wall, but they are too large to be prone to endocytosis. In the  
208 literature, it has been reported when testing microdevices with similar dimensions on Caco-2 cells that an interaction  
209 between the microdevices and the *in vitro* intestinal cell line was found (Chirra et al. 2014). Furthermore, it has been  
210 shown that microdevices with a similar diameter can stabilize amorphous forms of drug and avoid recrystallization as  
211 seen for amorphous forms without any confinement (Nielsen et al. 2012). It is found that the larger surface area of the  
212 microwells will enable large contact area with the intestinal epithelium if compared with spherical microparticles. This  
213 will be an advantage of the microwells and ensure suitable conditions for interactions between the microwells and the  
214 intestinal membrane in an *in vivo* situation.

215  
216 A simplified version of a screen-printing technique was employed for filling of the microwells with ASSF, the  
217 formation of which was confirmed by XRPD (data not shown). A representative example of a drug-filled microwell is  
218 shown in Fig 3, clearly illustrating that the process resulted in complete filling of microwells and a negligible  
219 distribution of drug on the edge of the well. Methods utilized to date for drug filling of micro devices (i.e.,  
220 microspotting and injection) have been limited to use in conjunction with aqueous drug solutions, and ideally with  
221 aqueous solutions containing 15-25 v/v% water-soluble polymer (Marizza et al. 2013). The use of such filling methods  
222 and conditions is time consuming, unsuitable for poorly water-soluble drugs, and furthermore impractical for large-  
223 scale filling of micro devices (Ahmed et al. 2002; Marizza et al. 2013). In contrast, the modified screen printing  
224 technique presented here constitutes a method where drug can be filled into devices in a relatively short time period,  
225 and can also be utilized for various types of drugs and drug formulations without the requirement for water solubility.

226  
227 For the majority of orally-administered drugs the main site of drug absorption is the upper part of the small intestine  
228 (only a few drugs show appreciable absorption through the stomach or colon) (Maher et al. 2008). Simulated gastric and  
229 intestinal media have been utilized in these *in vitro* studies to be as close to the *in vivo* situation as possible.  
230 Furthermore, it is known from the literature that the simulated media have an effect on the dissolution rate of ASSF  
231 compared to buffer, and therefore it is important in this situation to use the physiologically relevant media (Nielsen et

232 al. 2013a). Controlled drug delivery systems which prevent release in the gastric environment but facilitate its  
233 occurrence in the absorptive upper small intestinal region are therefore desirable, in order to provide for maximal oral  
234 drug bioavailability (Kleberg et al. 2010). In the current work, drug-filled microwells were spray coated with a layer of  
235 Eudragit® L100 in order to provide such drug protection and controlled release, and additionally to maximize drug  
236 retention within microwells until the absorptive environment of the small intestine was reached. Fig 4 illustrates the  
237 appearance of drug-filled microwells after coating with Eudragit® L100. The layer was found to be  $7.5\pm 0.7\ \mu\text{m}$   
238 ( $n=3\pm\text{SD}$ ) thick, with consistent thickness over the entire chip area.

239  
240 With respect to biorelevant characterization of drug release from the coated, drug-filled microwells,  $\mu$ -Diss profiler  
241 experiments demonstrated an absence of release in simulated gastric medium at pH 1.6 for 120 min (Fig 5). A rapid  
242 drug release and accompanying dissolution was however seen following a change to simulated intestinal medium (pH  
243 6.5), with 96% of the initially incorporated ASSF being released and dissolved within the 180 min period of exposure to  
244 such medium (Fig 5). The rapid release and dissolution of ASSF following the pH shift was expected given that  
245 Eudragit® L100 is known to dissolve above pH 6. Also, ASSF has been shown to have a very high dissolution rate in  
246 biorelevant intestinal medium (Nielsen et al. 2013a). The  $\mu$ -Diss profiler results therefore show that the Eudragit®  
247 coating is sufficiently thick to serve as an effective lid for the microwells, protecting the drug from being released at pH  
248 1.6, but providing no hindrance to a fast drug release at pH 6.5. This can be considered to translate to an effective  
249 protection of the drug by the Eudragit® coating together with the microwells in harsh conditions approximating those of  
250 the stomach, and to provide a controlled delivery of drug in an environment similar to that of the small intestine,  
251 allowing for effective drug absorption *in vivo*. With respect to furosemide, where a fast release is desirable, Eudragit®  
252 L100 constitutes an ideal candidate for microwell coating. In the case of other drug candidates where a prolonged  
253 release may be required, alternative polymers may be used as coating agents.

254  
255 In order to support the release results obtained using the  $\mu$ -Diss Profiler, flow through dissolution of ASSF from filled  
256 Eudragit®-coated microwells was studied in conjunction with UV imaging. UV imaging has earlier been shown to  
257 provide useful information regarding drug dissolution processes (Boetker et al. 2011; Ostergaard et al. 2014; Ostergaard  
258 et al. 2010), and further serves as an excellent visualization method of the dissolution of furosemide in biorelevant  
259 media (Gordon et al. 2013; Nielsen et al. 2013b). In the current work, UV imaging was employed to visualize the  
260 release and dissolution of ASSF from Eudragit® L100-coated microwells on a compressed timescale compared to the  
261 release studies conducted using a  $\mu$ -Diss profiler. UV imaging experiments demonstrated the same trend as that seen in  
262  $\mu$ -Diss profiler release studies. An absence of drug release and dissolution was noted in the gastric medium, with only a  
263 background level of absorbance evident in the absorbance maps (Fig 6A). An appreciable release of ASSF from the  
264 coated microwells could however already be observed 1 min following a switch of flow through dissolution medium to  
265 simulated intestinal medium and a re-equilibration of the dissolution cell (Fig 6B). This release was observed to  
266 increase (increased intensity of absorbance maps), after 5 min of exposure of microwells to a flow of biorelevant  
267 intestinal medium (Fig 6C). After 15 min of medium exposure the intensity of absorbance was noted to be decreasing  
268 (Fig 6D); this is likely due to an almost complete release of ASSF from the microwells, again reflecting the fact that  
269 ASSF has a fast dissolution rate at intestinal pH (Nielsen et al. 2013a; Nielsen et al. 2013b).

#### 270 271 **4 Conclusion**

272 Microwells consisting of the biodegradable polymer PLLA were successfully fabricated using a hot embossing process.  
273 Produced microwells had an inner diameter of 240  $\mu\text{m}$  and a depth of 100  $\mu\text{m}$ . It was possible to fill the microwells  
274 with ASSF using a screen printing technique which proved to be both effective and accurate in microwell filling,  
275 resulting in a minimal amount of drug being deposited around the edges of or external to the microwells. The developed  
276 technique is suggested to be a method that can be utilized for filling of all types of powder drug into micro devices.  
277 After coating of a Eudragit® L100 layer on the cavity of drug-filled microwells, drug release was studied using a  $\mu$ -Diss  
278 profiler as well as a UV imaging system in conjunction with biorelevant release media. Release experiments conducted  
279 using both techniques showed that the Eudragit® layer prevented drug release from microwells in biorelevant gastric  
280 medium, while an immediate release of the ASSF was seen in biorelevant intestinal medium. The developed Eudragit®-  
281 coated microwells therefore provide an effective drug protection and prevention of release in a gastric environment,

282 while allowing for rapid release in conditions approximating those of the small intestine. The fabricated PLLA  
283 microwells therefore show the ability to facilitate effective oral absorption of incorporated drug, demonstrating their  
284 significant future potential as oral drug delivery systems.

285

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290

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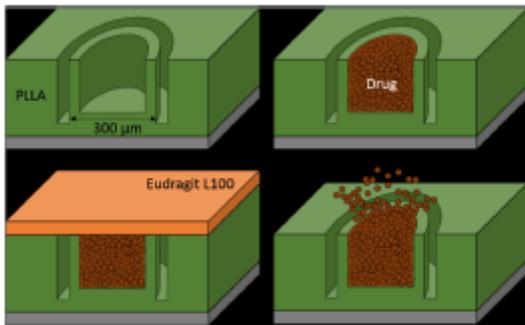
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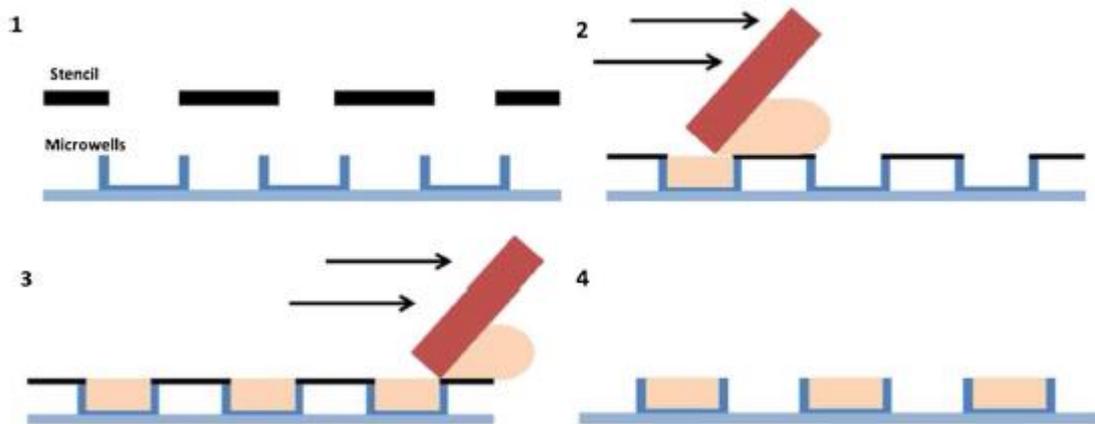
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328 **Figures and captions**  
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332 **Fig 1** Schematic drawing of the concept of the microwells as an oral drug delivery system: **a)** the biodegradable  
333 microwells fabricated from PLLA, **b)** filled with ASSF **c)** and subsequently spray coated with a lid of Eudragit® L100.  
334 **d)** at pH 6.5 (intestinal pH) ASSF is released from the microwells  
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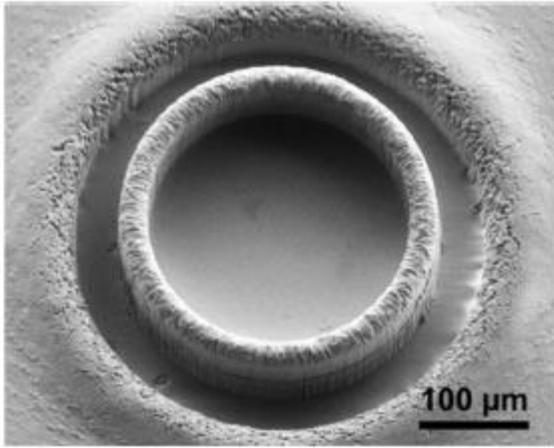
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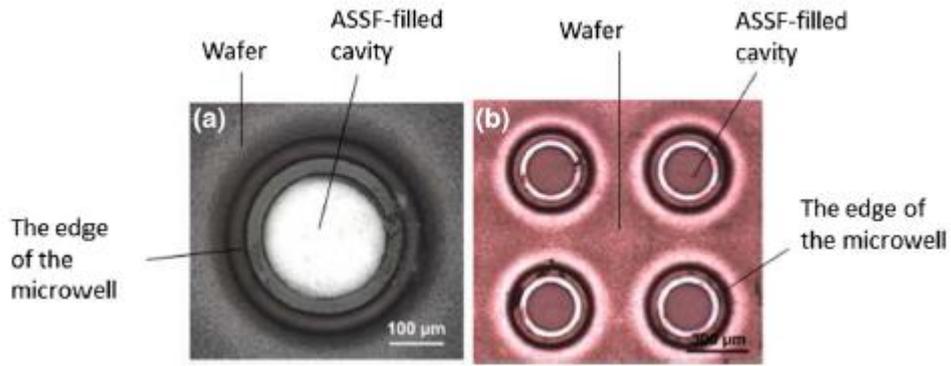
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**Fig 2** Graphical representation of the powder filling process of PLLA microwells using a modified screen printing process. 1. The fabricated stencil mask is aligned to the microwells. 2-3. ASSF is pressed into the microwells. 4. The stencil is removed, leaving PLLA microwells filled with powder drug

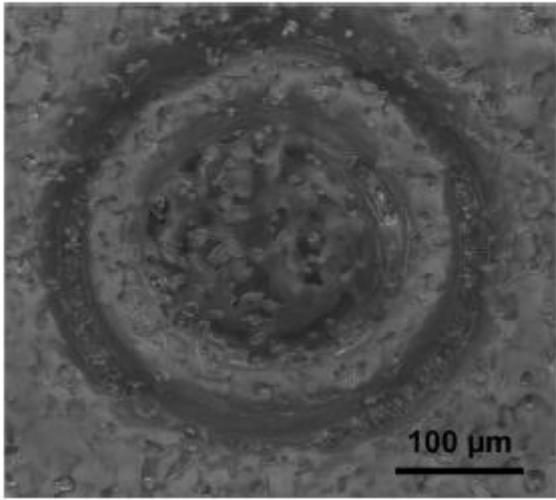


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**Fig 3** SEM image of a single microwell fabricated in PLLA by hot embossing

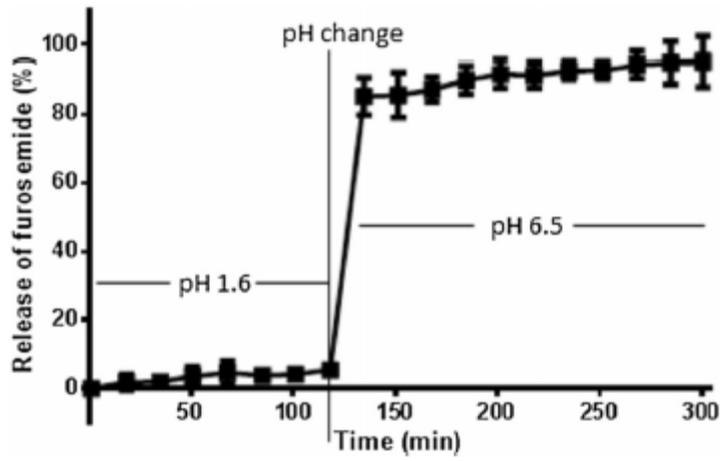


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349 **Fig 4** Representative optical microscope images (top view) of a PLLA microwell filled with ASSF  
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**Fig 5** A SEM image of drug-filled PLLA microwells spray coated with Eudragit® L-100

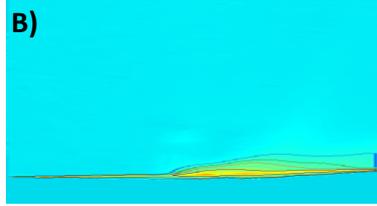


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 356 **Fig 6** Release profiles obtained from PLLA microwells filled with ASSF and coated with Eudragit® L100 in biorelevant  
 357 gastric medium pH 1.6 (0-120 min) and biorelevant intestinal medium pH 6.5 (120-300 min). Data shown represent the  
 358 mean of three replicates±SD  
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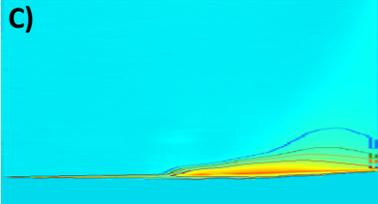
After 5 min in gastric media



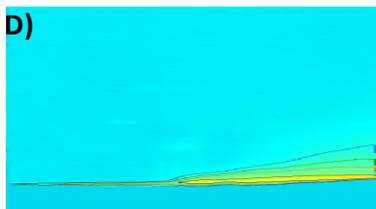
Intestinal Media - 1 min



Intestinal Media - 5 min



Intestinal Media - 15 min



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**Fig 7** Representative UV absorbance maps at 280 nm showing the release of ASSF from Eudragit® L100-coated PLLA microwells in **a)** simulated gastric medium (pH 1.6) after 5 min, and in simulated intestinal medium (pH 6.5) after **b)** 1 min, **c)** 5 min and **d)** 15 min. The contour lines correspond to absorbance values of: dark blue = 100 mAU, green = 150 mAU, orange = 200 mAU, light blue = 250 mAU, and brown = 300 mAU