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6 # Biodegradable microcontainers – towards real life applications of
7 # microfabricated systems for oral drug delivery

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12 Microfabrication techniques have been applied to develop micron-scale devices for oral drug delivery with a high degree
13 of control over size, shape and material composition. Recently, microcontainers have been introduced as a novel approach
14 to obtain unidirectional release to avoid luminal drug loss, enhance drug permeation, protect drug payload from the harsh
15 environment of the stomach, and explore the ability for targeted drug delivery. However, in order to eventually pave the
16 way for real life applications of these microfabricated drug delivery systems, it is necessary to fabricate them in
17 biodegradable materials approved for similar applications and with strategies that potentially allow for large scale
18 production. In this study, we for the first time evaluate biodegradable microcontainers for oral drug delivery. Asymmetric
19 poly-ε-caprolactone (PCL) microcontainers with a diameter of 300 μm and a volume of 2.7 nL are fabricated with a novel
20 single-step fabrication process. The microcontainers are loaded with the model drug paracetamol and coated with an
21 enteric pH-sensitive Eudragit® S100 coating to protect the drug until it reaches the desired location in the small intestine.
22 *In vitro* dissolution studies are performed to assess the drug load and release profile of the PCL microcontainers. Finally, *in*
23 *vivo* studies in rats showed a higher bioavailability compared to conventional dosage forms and confirm the potential of
24 biodegradable microcontainers for oral drug delivery.

1. Introduction

Among the various conventional ways of drug administration, oral delivery of pharmaceuticals is the preferred route as it offers several advantages. It is non-invasive, provides high patient compliance and is associated with low manufacturing costs. However, the majority of new drug entities entering the market are poorly water soluble which results in a low bioavailability.[1,2] Also, for peptide and protein drugs, oral delivery is a challenge as they can potentially degrade, before reaching their target location in the intestine e.g. due to the low pH in the stomach or the presence of bacteria or enzymes.[3,4]

In the past decades, microfabricated drug delivery systems have been proposed to overcome some of these major challenges in oral drug delivery.[5-8] For this purpose, well-established microfabrication techniques from the semiconductor industry have been applied to reproducibly manufacture microcontainers with precisely controlled dimensions and shapes.[9–11] These devices could potentially provide unidirectional release, control of drug release kinetics and have the ability for targeted delivery of pharmaceuticals in the gastrointestinal tract (GIT).[12,13] The concept of these microcontainers is illustrated in **Figure 1**. The geometrical structure is cylindrical (**Figure 1A**) and provides a large surface area that can attach to the mucus layer of the intestinal wall. Drugs in various forms such as powder, liquid or distributed in a drug-polymer matrix have been loaded into the microcontainers (**Figure 1B**).[14–18] To control the release kinetics and to increase the oral bioavailability of the drug, functional coatings such as a pH-sensitive polymer lids have been applied (**Figure 1C**) [19,20]. When the microcontainers reach the desired location in the GIT, the coating is dissolved and the drug is released followed by intestinal absorption (**Figure 1D-E**). Several studies have shown that the microfabricated containers can potentially increase the residence time in the intestine and thereby the oral bioavailability of the loaded drug.[18,19]
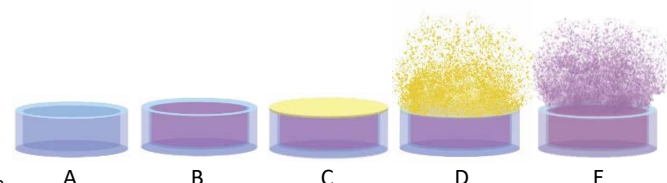
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Figure 1. Schematic representation of the microcontainer concept. A) the microcontainers are fabricated with a bottom and walls, B) a desired drug or formulation is loaded into the microcontainer cavities, C) a coating is applied in order to protect the drug until release is desired, D) when the microcontainers reach the desired area in the intestine, the lids are dissolved and E) the drug is released

The first microfabricated drug delivery systems were produced in materials conventionally used in microfabrication such as silicon[22], poly(methyl methacrylate) (PMMA)[10], and photoresists.[23] However, in the last years there has been a high impetus to fabricate microdevices in biocompatible and biodegradable polymers.[18,19,24,25] DeSimone *et al.* have introduced the PRINT technique (Particle Replication in Non-Wetting Templates) which uses molding in a polymer stamp to produce micron-scale and sub-micron-scale structures.[26,27] Furthermore, Guan *et al.* have described a process to produce foldable hydrogels for drug delivery applications.[6] In our center, hot punching has been developed as a simple, low cost and scalable process suitable for the fabrication of individual polymeric microparticles with high structural replication fidelity.[25] In conventional hot embossing, a polymer film is deposited on a carrier substrate and patterned using a stamp. The significant difference of hot punching compared to hot embossing is the introduction of an elastic layer below the actual polymer device layer, allowing discrete particles to be punched out due to penetration of the residual layer.[25] For the fabrication of microcontainers, hot punching addresses many of the fundamental shortcomings of other methods. It is potentially scalable, enabling the fabrication of large numbers of discrete polymer microcontainers with high aspect ratios using biodegradable materials suitable for oral drug delivery. The hot punched microcontainers are obtained in ordered arrays solely defined by the stamp design and with the open side of the reservoir pointing upwards which facilitates their handling and further processing such as drug loading. As a major limitation, the fabrication of Poly(L-lactic acid) (PLLA) microcontainers, demonstrated earlier, requires multiple processing steps such as spin coating of several polymer layers for the hot punching process.[28] However, spin coating is inherently a batch process, requires preparation of polymer solutions with organic solvents such as acetone or dichloromethane (DCM), and further results in considerable material waste. Furthermore, harvesting of the microcontainers from the carrier substrate has either been based on manual removal or required additional bonding steps for removal from the stamp, which is not suitable for large sample volumes.

Due to the small dimension of the microcontainers, one of the major challenges is to load drug into their cavities. The optimal method should allow for parallel loading of a large number of microcontainers with identical amounts of drug while providing minimal drug waste. In the past, various methods for drug loading into reservoir-based drug delivery systems have been proposed.[7] Ainslie *et al.* proposed UV crosslinking of hydrogel matrices with drug. However, the amount of drug that is loaded with this approach is very restricted.[11,12] Alternatively, hot punching in a spin-coated drug-polymer film or supercritical CO_2 impregnation of microcontainers filled with polymer have been demonstrated.[20,29,30] In all these methods, solubility of the drug in the polymer matrix or in the supercritical CO_2 is required.

Furthermore, the polymer matrix itself will occupy a considerable part of the microcontainer volume, thereby reducing the amount of drug that can be loaded. Typically, drugs are available in powder form acquired from commercial suppliers or prepared as microparticles e.g. using spray drying. Therefore, recently powder embossing has been introduced for reproducible loading of arrays of microcontainers with drug powder.[31] This method provides an excellent loading efficiency, homogeneity, and reproducibility. However, it involves the manual alignment of a shadow mask to the cavity of the microcontainers. This approach is suitable for small experimental studies but not applicable for fabrication of large amounts of drug delivery devices.

Here, we for the first time evaluate the application of biodegradable microcontainers for oral drug delivery. For this purpose, we have developed a novel approach for fabrication of microcontainers in the biocompatible and biodegradable polymer poly-ε-caprolactone (PCL) using a single-step of hot punching. Spin coating of polymer layers on solid carrier substrates was replaced by simple assembly of compression molded polymer films prior to a single step of simultaneous thermal bonding and patterning based on hot punching. This process potentially allows for continuous fabrication of the polymeric devices without the need of organic solvents or expensive batch processing steps. Furthermore, a fast and precise loading of the microcontainer cavities with the model drug paracetamol is demonstrated. Paracetamol was chosen as model drug for this study as it is absorbed in the intestine after oral administration with an oral bioavailability between 70-90 % and there is no absorption though the stomach[32][33]. Also, paracetamol is highly water soluble and it is therefore easily released from the microcontainers in aqueous media[34][35]. For the purpose of loading the model drug into microcontainers, a modified powder embossing method is implemented where the already existing residual polymer film between the microcontainers replaces the need for the alignment of a shadow mask. The drug-loaded PCL microcontainers are covered by a pH-sensitive coating of Eudragit® S100 applied by spray coating. The microcontainer harvesting step is facilitated by a water soluble substrate of poly(vinylalcohol) (PVA) which is simply dissolved in aqueous medium after microcontainer preparation. The microfabricated drug delivery system is evaluated both *in vitro* and *in vivo* to demonstrate the potential for efficient oral drug delivery.

2. Results and discussion

The novel fabrication process for biodegradable microcontainers is illustrated in **Figure 2A**. For the proof of concept, PCL was selected as device material and cylindrical microcontainers with a nominal inner diameter of 230 μm, a height of 90 μm and a reservoir depth of 65 μm were designed and fabricated. These dimensions were selected because similar microdevices earlier provided promising drug release kinetics *in vitro* and improvement of oral bioavailability *in vivo*.[19–21]
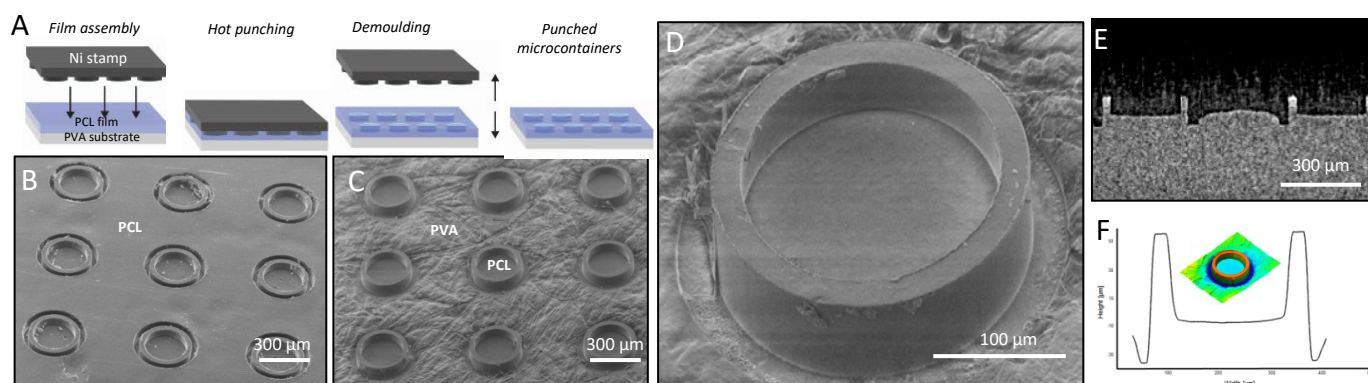
Figure 2. A) Schematic overview of fabrication process: A compression molded PCL film and a PVA substrate are assembled. Hot punching is performed by applying pressure and heat with a Ni stamp. Lastly, a demolding step of the Ni stamp is completed, leading to separation of PCL microcontainers from the surrounding film, B) PCL microcontainer array and surrounding PCL film, C) PCL microcontainer array after removal of the surrounding film, D) Close-up of a single PCL microcontainer on the PVA substrate, E) Cross-sections acquired by XµCT through the empty microcontainers, F) Optical profile curve and 3D rendering of one single unit for the PCL microcontainer

In this work, compression molding was used to prepare thin PCL device films and PVA substrates. In this process two hot plates function as the molds compressing preheated PCL or PVA pellets into thin films (**Supplementary information, S1**). This method was introduced to achieve thin and uniform polymer films without the use of solvents, with no waste of material, and to demonstrate the possibility to move from a batch process, such as spin coating, to continuous film preparation using e.g. film extrusion.

The process started with the assembly of a PCL device film and a PVA substrate. The device film was then molded by a robust Ni stamp and finally punched due to the backpressure exerted by the PVA substrate. After the hot punching process was completed, the microcontainers were physically separated from the surrounding PCL film but remained as separate units attached to the underlying PVA substrate.

A series of optimization steps were carried out to achieve the desired thickness of the PCL film using compression molding. By varying the processing parameters such as the initial amount of polymer (0.5-3 g), temperature (60-80 °C) and the hydraulic pressure (20-50 bars), it was possible to adjust the film thickness in the range of 82-103 µm. The maximum depth of the structures on the Ni stamp used in this study was approximately 90 µm (**Supplementary information, S2**). Therefore, a slightly lower thickness of the PCL film ensured that the Ni stamp was able to fully penetrate it and reach the PVA substrate during the hot punching process. At the same time, it should ensure complete filling of the stamp features with PCL.

PVA was chosen as the substrate due to its high solubility in aqueous medium, high tensile strength and flexibility.[36] The thickness of the PVA substrate had to be sufficiently high to provide mechanical stability during the punching process and, at the same time, as low as possible to allow its dissolution in aqueous medium in the shortest possible time. The latter was required for harvesting of the microcontainers after completed fabrication where a shorter immersion time in the medium reduces potential loss of drug. In order to control the thickness of the PVA layer, the initial amount of polymer (5-35g) and the hydraulic pressure (6-60 bars) were adjusted. Thicknesses in the range of 500-1500 µm were achieved

and the final optimal thickness was chosen to be 525 ± 17 µm (SD, n=3) as thinner substrates showed mechanical instability and started cracking during the demolding step.

The fabrication of microcontainers was carried out by assembling the Ni stamp, the PCL device layer and the PVA substrate. In a single step, 1,600 units were punched out arranged in four arrays, each consisting of 20x20 microcontainers. The optimized temperature for the hot punching process was 70°C. At this temperature, the PVA substrate still presented mechanical stability and elastic-like properties required for punching of the residual layer due to a glass transition temperature (T_g) for PVA of 85°C. Furthermore, the hot punching temperature was slightly higher than the melting temperature (T_m) for PCL of 60°C and therefore the PCL device film was in a melted state. Initial optimization was performed by varying the holding time (500-1200 s) and the hydraulic pressure (4-20 bars). With the optimized parameters, PCL microcontainers were successfully punched out and separated from the surrounding PCL film as seen in **Figure 2B**. The PCL microcontainers adhered well to the PVA substrate as visualized after mechanical removal of the surrounding PCL film in **Figure 2C-D** and as illustrated by the x-ray microtomography (X-µCT) image (**Figure 2E**). On one hand demolding was possible due to the low surface energy of the Nickel stamp coated with a monolayer of perfluorodexyltrichlorosilane (FDTS). On the other hand, the polar OH groups of PVA provided excellent compatibility with the ester groups of PCL leading to exceptional adhesion of PCL microcontainers to PVA. The hot punching with the optimized parameters resulted in replication of all microcontainers in a single fabrication step and a total process time of 14 min including holding and embossing time. This was a significant improvement compared to preliminary experiments carried out using a PDMS penetration layer (results not shown). The inner and outer height of the PCL microcontainers were 64.1±1.0 µm (SD, n = 3) and 92.0±1.5 µm (SD, n=3), respectively. The inner diameter was 230.5±2.2 µm (SD, n=3) (**Figure 2F**), which is very close to the nominal diameter.
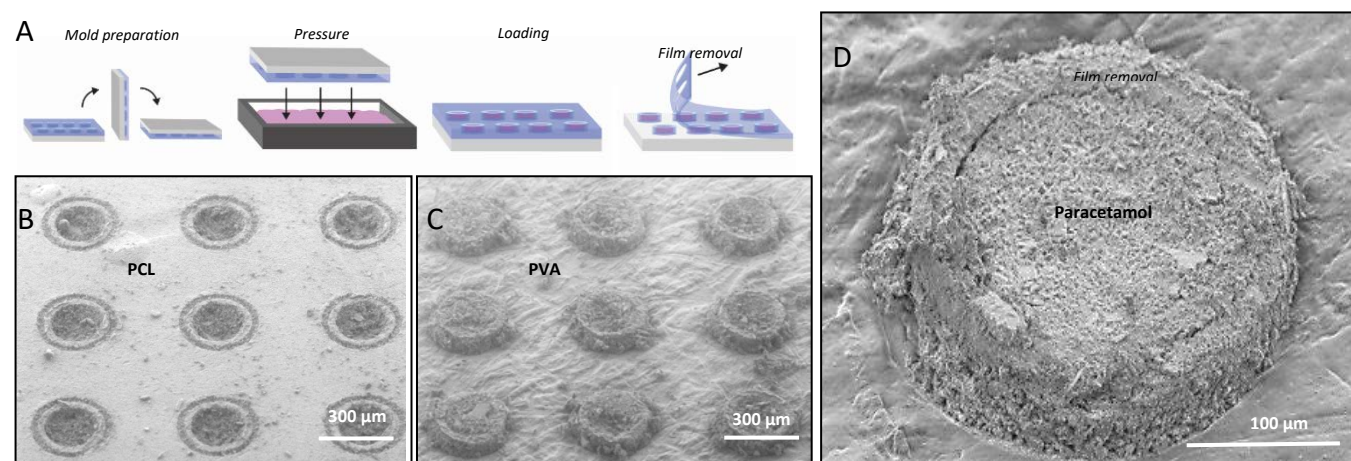
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Figure 3. A) Schematic process overview of the drug loading in microcontainers: The PCL microcontainers with the surrounding film are used as mold in the powder embossing process. Paracetamol powder is placed in a holder and pressure is applied. After the pressure is released, the microcontainers are loaded with paracetamol and shadow mask is removed, B) Microcontainer array after the loading process before removal of the surrounding film, C) Microcontainers after the loading process and after removal of the surrounding PCL film, D) Close-up of a single loaded PCL microcontainer

For fabrication of microcontainers, the hot punching process presents several benefits, such as the penetration of the residual layer during a single thermal processing step, no formation of residues and no requirement for additional equipment compared to similar attempts using reactive ion etching or laser machining techniques.[33,34] Furthermore, the resulting microcontainers are placed in ordered arrays solely defined by the stamp design and with the cavity of the reservoir pointing upwards which facilitates their handling and further steps such as drug loading.

2.2 Loading of drug into the PCL microcontainers

The microcontainer arrays with empty cavities were loaded with paracetamol drug powder. In similar studies, this step required the manual alignment of a shadow mask.[31] Here, the surrounding PCL film remaining after the punching process (**Figure 2B**) was used as a stencil to avoid drug deposition in between the microcontainers. **Figure 3A** shows the overall concept of the drug loading method. The PVA substrate containing the PCL microcontainers and the surrounding PCL film was used as a stamp for embossing into drug powder (**Figure 3B**). After the embossing process, the surrounding PCL film was removed, limiting drug loading to the container reservoirs. Up to four arrays of 20x20 devices were simultaneously embossed into paracetamol powder both in as purchased and grinded forms. Due to the coarse structure of the powder, grinding allowed a more uniform, complete and denser loading (see **Supplementary information S3** for SEM images of un-grinded paracetamol loading) of all the microcontainers in the array. As seen in **Figure 3C** and **D**, drug residues between the microcontainers were minimal after removal of the surrounding PCL film.

A successful loading was characterized by drug inside all container cavities. The powder filling required only a few seconds to be performed and resulted in all 1,600 units loaded in one single step. The drug loading was evaluated using microdissolution with arrays of 20x20 devices (**Supplementary information S4**). Based on the measured dimensions, each PCL microcontainer has a cavity of 2.7 nL and could therefore theoretically contain up to 3.4 µm

paracetamol. The measured amount of paracetamol loaded in a single microcontainer was 2.4±0.1 µg (SD, n=5), corresponding to a loading efficiency of 71 %. The lower actual amount of drug loaded in the microcontainers demonstrates that despite the powder embossing there is still a considerable amount of free volume in each cavity.

2.3 Enteric coating and harvesting of the drug-loaded PCL microcontainers

In order to apply the microcontainers for drug delivery in the small intestine, it is desirable that the drug is protected during passage through the stomach. Therefore, the integration of a pH-sensitive coating that only dissolves when the microcontainers reach the small intestine (approximately pH 7) is required. Enteric polymers have previously been widely investigated for drug delivery systems to overcome the acidic barriers in the stomach. In particular, Eudragit® polymers have been successfully employed in many studies for oral dosage forms.[39] Here, we have combined the advantages of microfabricated drug delivery devices, including controllable size and shape and unidirectional release with the pH dependent enteric coating which provides a unique system in comparison to existing pH responsive drug delivery systems[39][40]. Recently, spray coating has been introduced for the deposition of Eudragit® films on SU-8 microcontainers and biopolymer microwells loaded with drug.[20][22] . These polymeric lids are stable in simulated gastric medium (pH 1.6) and dissolve upon immersion in simulated intestinal medium (pH 7.5), triggering the release of the drug. The advantage of this method is that spray coating allows applying a uniform coating on large arrays of microcontainers.

In this work, we implemented spray coating of Eudragit® S100 on PCL microcontainers (**Figure 4A**). The pH-sensitive coating applied on top of the microcontainers had a thickness of 38±8 µm (SD, n=5). X-µCT (**Supplementary information, S5**) and SEM images were used to assess the morphology of the coating after its deposition on the cavity of the microcontainers. **Figure 4B** shows that the coating was uniform and smooth and that the drug-loaded microcontainers were completely covered. For the harvesting of the PCL microcontainers, the PVA substrate was dissolved. The dissolution
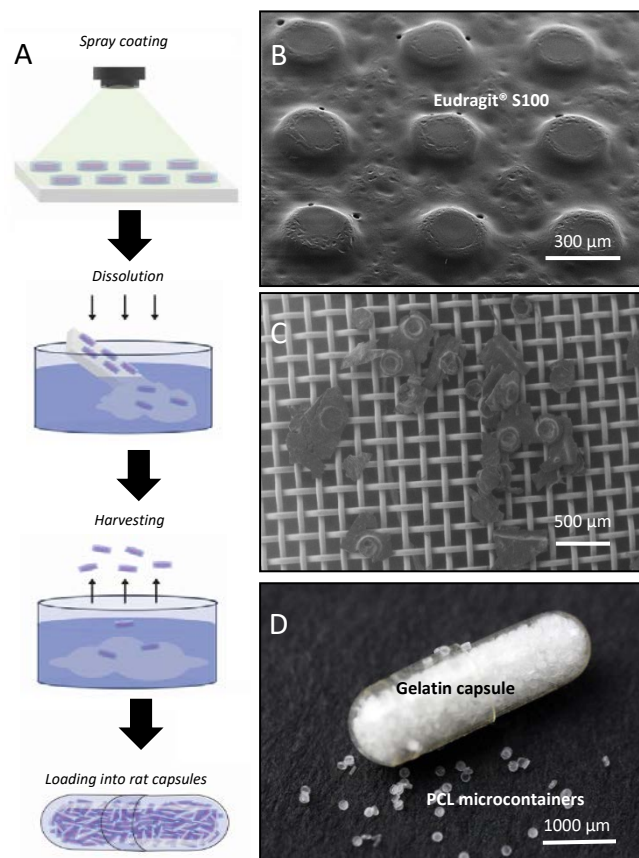
Figure 4. A) Schematic process overview for coating and harvesting microcontainers: PCL microcontainers on PVA substrate are coated with the pH-sensitive polymer of Eudragit® S100. For harvesting microcontainers and dissolving the PVA substrate, the substrate is poured into an aqueous solution. The microcontainers are harvested using a grid and then loaded into gelatin rat capsules B) Microcontainer arrays with an Eudragit® S100 coating, C) Harvested microcontainers on a stainless steel grid, D) Rat gelatin capsule loaded with empty microcontainers

of the sacrificial PVA substrate was achieved by immersion in acidic aqueous medium (pH 3) for 40 min. Subsequently, the free-floating microcontainers were harvested using a stainless steel filter mesh (**Figure 4C**) and loaded in rat capsules (**Figure 4D**).

2.4 *In vitro* release of paracetamol from coated PCL microcontainers

In vitro release studies in a microdissolution profiler were used as a preparative step before the final *in vivo* studies in order to i) experimentally quantify the amount of drug in each rat capsule and ii) evaluate the performance of the enteric pH-sensitive coating applied on the cavity of the drug-loaded microcontainers.

Rat capsules were prepared for the *in vitro* studies and each of them were filled with approximately 800 microcontainers. To evaluate the drug release from microcontainers in gastric and intestinal conditions, the *in vitro* dissolution was completed in two simulated media with a volume of 10 mL. The release of paracetamol was first measured for 30 min in intestinal medium with pH 1.6. In this period, 0.25±0.60 mg paracetamol were

released from the microcontainers. This was followed by an investigation of drug release from the gastric medium for 150 min simulating the transit time in the small intestine. **Figure 5** shows that a burst-like release with a significant immediate release of paracetamol was measured for the coated microcontainers. A fast release in the first hour was observed, after which the release curve started saturating. The final amount of drug released after 180 min showed that each capsule was loaded with 0.72 ± 0.04 mg paracetamol, which is sufficient drug for *in vivo* studies. At the same time, this value was lower than expected considering the initial amount of drug loaded in the microcontainers. It is assumed that drug loss mainly occurred during the spray coating of the lids and eventually also during the harvesting of the devices.
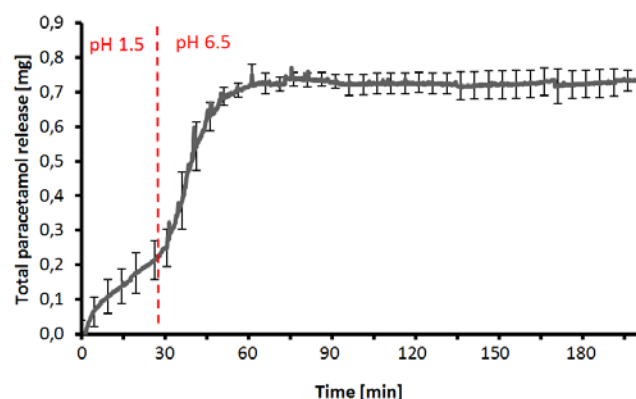

Figure 5. Drug release profiles over time obtained from one capsule of microcontainers filled with paracetamol and coated with Eudragit® S100 in gastric medium at pH 1.6 (from 0 to 30 min) and intestinal medium pH 7.5 (30-180 min). Data are presented as mean ± SD (n=5).
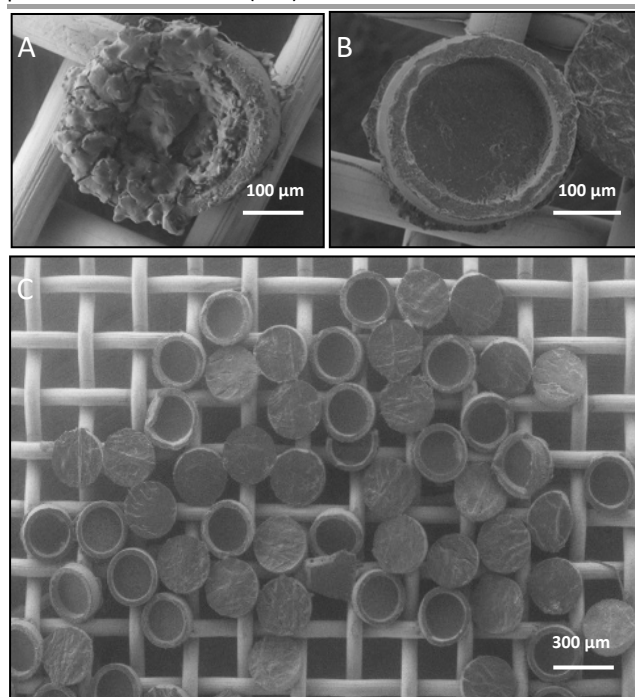

Figure 6. A) Microcontainer after immersion in the gastric medium for 30 min, B) close-up of a single microcontainer harvested after 150 min in intestinal media, C) Microcontainers harvested after 150 min in intestinal medium

To verify that the entire drug content was released from the capsules after the dissolution study, the microcontainers were investigated in SEM. After immersion in gastric medium for 30 min (**Figure 6A**) the enteric coating was still intact and thus the drug was expected to remain inside the microcontainers. For intestinal medium, **Figure 6B** and **C** confirm that the microcontainers were completely empty after 180 min.

2.5 Oral pharmacokinetic (PK) study in rats

In order to study the pharmacokinetic (PK) profile of paracetamol when using PCL microcontainers as vehicle for oral drug delivery, an *in vivo* study in rats was performed. Rat capsules filled with PCL microcontainers, loaded with paracetamol and coated with enteric coating of Eudragit® S100, were administered to rats using oral gavage. As a control, rat capsules filled with paracetamol powder and coated with Eudragit® S100 were administered. **Figure 7** displays the plasma concentration of paracetamol over time after oral dosing of the drug-loaded PCL microcontainers and the control formulation.
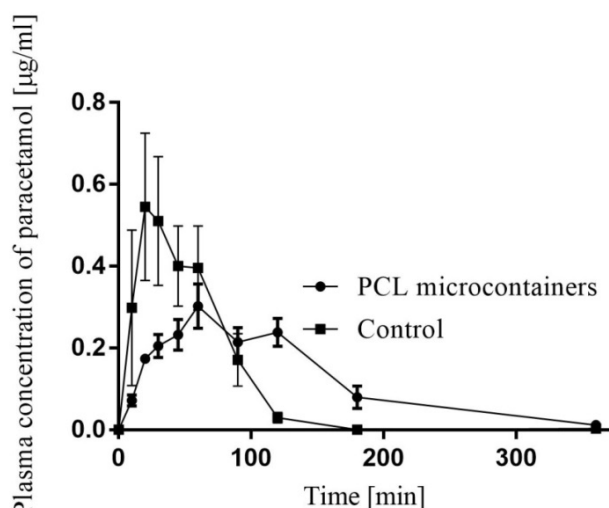

Figure 7. Plasma concentrations of paracetamol after oral dosing to rats of either PCL microcontainers filled with paracetamol, coated with Eudragit® S100 and filled into gelatine capsules or control capsules filled with paracetamol and coated with Eudragit® S100. The data is presented as mean ± SEM with n = 7 for PCL microcontainers and n = 8 for control capsules.

Table 1 presents the non-compartmental analysis of the PK parameters. The plasma concentration versus time curve shows a fast absorption of paracetamol over the first 90 min for the control formulation. For the PCL microcontainers, it was noted that the profile never reached the same maximum plasma concentrations as the control formulation. This is reflected in the maximum plasma concentration (C_{max}), which for PCL microcontainers was found to be 0.3 ± 0.1 µg/mL, whereas it for the control was significantly higher being 0.9 ± 0.5 µg/mL (p-value 0.007). The time until C_{max} (T_{max}) for PCL microcontainers and the control was 59 ± 31 min and 38 ± 26 min, respectively, and was not significantly different (p-value 0.2371). Nevertheless, it still showed a tendency for delayed and sustained absorption of paracetamol released from the PCL microcontainers. The sustained absorption might be due to a mucus adhesive effect leading to a prolonged retention of the PCL microcontainers in the absorptive area of the small intestine. The

PCL microcontainer formulation kept the plasma concentration of paracetamol at a constant level for a longer time period compared to the control. This resulted in an Area Under the Curve ($AUC_{0-360 \, min}$) for paracetamol of 60 ± 33 µg·min/ml for the PCL microcontainers, and of 39 ± 18 µg·min/ml for the control. No significant difference was observed (p-value 0.5973), but the results indicate a trend towards a higher $AUC_{0-360 \, min}$ for the PCL microcontainers (**Table 1**).

Table 1. Non-compartmental pharmacokinetic parameters of paracetamol after oral dosing to rats of either PCL microcontainers or the control formulation. Data is presented as mean ± SEM.

	PCL microcontainers (n=7)	Control capsules (n=8)
T_{max} [min]	58.6 ± 31.0	38.1 ± 26.2
C_{max} [µg/mL]	0.3 ± 0.1*	0.9 ± 0.5*
AUG_{total} [µg-min/mL]	60.6 ± 33.5	38.8 ± 17.8

*Significant difference after unpaired t-test variance tested with F-test showing significant difference- $p<0.05$

The relative bioavailability was calculated for the paracetamol in the biodegradable PCL microcontainer formulation in relation to the control formulation, and it was found to be 166 ± 116 %. When examining the small intestine and caecum of three rats euthanized 6 h after dosing by stereomicroscopy (Supplementary information, S6), no PCL microcontainers could be localized. The absence of PCL microcontainers implies that they passed the small intestine at this time.

The *in vivo* study demonstrates that the biodegradable microcontainers display the same promising features for oral drug delivery as the ones earlier discussed for non-biodegradable microcontainers.[13,20,35] Firstly, the delayed and sustained absorption observed for the PCL microcontainers is similar to results obtained in *in vivo* rat studies with SU-8 microcontainers filled with amorphous furosemide salt.[21] Secondly, *in vivo* studies with drug loaded SU-8 or poly(methyl methacrylate) microdevices also resulted in a higher AUC and relative bioavailability compared to similar controls.[15,20,21]

3. Experimental section

3.1 Fabrication poly-ε-caprolactone microcontainers: PVA (Mowiflex C17, Kuraray) substrates and PCL (M_n=80,000 g mol^{-1}, Sigma Aldrich) device films were prepared by compression molding by a hot embosser (Collin® Press, 300 SV) using the parameters presented in **Table 2**.

Table 2. Parameters for fabrication of compression molded polyvinyl alcohol substrates and poly-ε-caprolactone device layer

Material	Amount [g]	Compression time [min]	Temperature [°C]	Cooling ramp [K/min]	Platen pressure [bar]

PVA	5	17	150	20	60
PCL	0.5	25	70	20	19

The compression molded PVA substrates were laser cut (Epilog laser mini, 30 W) in squares of 28x28 mm to fit 4x400 microcontainers. The PCL sheets were cut in 28x28 mm by a scalpel. For the hot punching process, the PCL device film was assembled on top of the PVA substrate. The device film was molded and punched by a robust Nickel stamp (70°C, 500 sec., platen pressure at 4 bars). Nickel stamps were fabricated using dry etching and electroplating in a similar manner as described by Petersen et al.[42] After the punching process was finished, the temperature was decreased with a cooling ramp of 20°C min^{-1} to room temperature. Then the stamp was demolded from the polymers. The microdevices were visualized using a Scanning Electron Microscope (SEM). All SEM micrographs were taken by a TM3030Plus Tabletop Microscope (Hitachi, Germany) with a voltage of 15 keV using the SE detector. Optical profiler measurements were performed at DTU Nanolab with PLu Neox 3D optical profilometer (Sensofar, Spain). 20X VSI measurements were conducted on the sample. Stylus profiler (Dektak XTA) measurements were performed to ensure correct height determination by VSI. The data was analyzed using the free SPM data analysis software Gwyddion and the data was levelled with respect to the indentation. Heights were determined based on profiles extracted across the center of the microcontainers. VSI scans were performed near the center and in each of the four corners of the chip. More detailed scans were also made for use in 3D rendering.

3.2 Loading of drug into the microcontainers: For the loading of microcontainers after container fabrication, the surrounding PCL film was used as a stencil. An electrical compressive press (MTI Corporation, YLI-1-4TA) was used for the drug loading of the microcontainers. For uniform transfer of the powder to the microcontainers, the paracetamol powder was placed in a micro milled recess with sidelength of 15 mm and a depth of 1 mm. A constant pressure of 8x10^{-1} T was applied for 30 sec. The powder was embossed inside the container cavities after which pressure and surrounding film were gently removed. The remaining powder was reused for loading of following samples.

3.3 Enteric coating deposition: The microcontainers were spray-coated with the pH-sensitive polymer Eudragit® S100 (Evonik, Germany). The solution was prepared mixing 1 % w/v Eudragit® S100 in isopropanol (Sigma Aldrich, USA). 5 % w/w of dibutyl sebacate (Sigma Aldrich, USA) in relation to the polymer was added as plastizier. 2 % v/v of MilliQ deionized water was added to the final volume. The solution was sprayed over a chip consisting of 400 drug-loaded microcontainers using an ultrasonic spray coater equipped with an accumist nozzle operating at 120 kHz (Sono-Tek USA). During the procedure, the flow rate was kept at 0.1 mL/min, together with a 1.3 W generator power. The shaping air was set to 0.02 bars, and the speed of the nozzle was maintained at 10 mm sec^{-1}, keeping a distance between the tip and the sample of 5 cm. Two alternating spray paths were employed having an offset of 1 mm, resulting in a total of 60 passages. The temperature during the spray coating process was kept at 40°C.
For thickness measurements, clean silicon wafer chips were spray coated with the same Eudragit® solution and the same settings. The thickness measurements were performed with a KLA-Tencor Alpha-Step IQ stylus profilometer (Milpitas, USA) with a scan speed of 50 µm s^{-1} and force of 15.3 mg. Each chip was measured in three different areas (middle and each side).

3.4 Morphology characterization: The samples as described in **Section 4.1** were investigated using a commercial X-ray microtomography system (Zeiss Xradia 410 versa, Germany). The system has an X-ray source operated in reflection geometry, a working high voltage between 40 kV and 150 kV and with a power up to 10 W. Samples were mounted on a flat seam in order to enable alignment to ensure that they were within the field of view in the horizontal plane of the detector. A source voltage of 60 kV and power of 10 W were used for all measurements in combination with different resolutions settings. Each sample was imaged first with a low resolution using the Large Field of View objective (from 14.5 µm to 19.5 µm pixel size for different samples and a collection time of 1.5 h, using 1601 projection Images to cover 360° rotation) in order to observe most of the sample. Then, an area of interest was selected for further investigation with a higher resolution (4.03 µm pixel size and a collection time of 3.5 h with 1601 projection images covering 360° rotation) to properly investigate the loading of 25 microcontainers using the '4x' objective. Tomographic data were reconstructed using the commercial software available for the system. The reconstruction software is based on the FDK method which is a filtered back projection algorithm.[43]

3.5: Preparation of rat capsules: Four chips (4x400) of microcontainers were loaded simultaneously and each chip was then coated individually as described above. The solubilization of the sacrificial PVA layer was obtained by soaking the four chips at a time into 600 mL acidic media (pH 3). After 40 min, the microcontainers were filtered with a stainless steel filter with a mesh opening of 213 µm and thickness of 178 µm (Spectra/Mesh® Woven Filters, Fisher Scientific, Denmark). The microcontainers were dried at 37°C for 1 h. Gelatin capsules (Torpac®, size 9, USA) were filled with individualized microcontainers and weighted before and after filling. The amount of drug inside each rat capsule was estimated based on the microdissolution data. The capsules were weighted before and after loading with microcontainers, and drug loading was normalized to the mean of the total release from the dissolution curve. As controls, capsules were loaded with 0.75 ± 0.006 mg of grinded paracetamol powder with an amount corresponding to the estimated amount inside the container-loaded capsules. Subsequently, the capsules were coated with a solution of 5 % w/v Eudragit® S100 in isopropanol and 5 % w/w dibutyl sebacate in relation to the polymer. The capsules were coated by dipping half of it into the coating solution and dried for 15 min before coating the other half.

3.6: In vitro study: The in vitro release of paracetamol from the microcontainers was tested using a µ-Diss profiler (pION INC, USA) in a similar setup as described elsewhere.[22] Experiments were carried out at 37°C employing a stirring rate of 100 rpm. The path length of the in situ UV probes was 1 mm, and each channel of the profiler was calibrated with its own standard curve prior to the experiments. The loaded capsules containing either microcontainers or the control powder formulation were poured in 10 mL Fasted State Simulated Gastric Fluid (FaSSGF pH 1.6, Biorelevant®, UK) solution after starting the experiment. The experiment was carried out for 30 min and then the microcontainers were filtered out using a stainless steel grid. Then the medium was changed to 10 mL Fasted State Simulated

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Intestinal Fluid (FaSSIF pH 7.5, Biorelevant®, UK) solution in each vial and the experiment was carried out for 150 min.

3.7: Oral PK study in rats: All animal experiments in current study were performed at the Department of Pharmacy, University of Copenhagen, under the license number 2016-15-0201-00892 in agreement with Danish laws regulating experiments on animals and EU Directive 2010/63/EU. Sprague Dawley (SD) male rats of an age of 7 weeks (Janvier, France) were acclimatized for 7 days with ad libitum access to water and standard feed. They had a switched light/dark cycle of 12/12 h, in a relative humidity of 55 ± 10 % and a temperature of 22 ± 1 °C. The rats were fasted for 12 h prior to dosing with ad libitum access to water and on the day of the study, the weight of the rats was 310 ± 8 g. The rats were randomly dosed either with one capsule of loaded PCL microcontainers or one capsule containing the control. Each capsule was filled with approximately 800 paracetamol-filled, Eudragit® S100 coated PCL microcontainers, corresponding to a dose of 2.8 ± 0.4 mg kg^{-1} paracetamol. For the control, capsules coated with Eudragit® S100 filled with 2.4 ± 0.0 mg kg^{-1} crystalline paracetamol. Each formulation was dosed to 8 rats, and the capsules were dosed by oral gavage with a polyurethrane feeding tube (Instech laboratories Inc., Plymouth Meeting, U.S.). Blood samples (200 μL per sampling) were retrieved through the tail vein at 0, 10, 20, 30, 45, 60, 90, 120, 180 and 360 min and collected in ethylenediaminetetraacetic acid (EDTA) tripotassium salt dehydrate coated Microvette®-tubes (Sarstedt, Sweden) and centrifuged at 4°C at 10,000 rpm for 10 min to obtain the plasma. The plasma was collected and immediately stored at -18°C until high-performance liquid chromatography (HPLC) analysis. The rats were euthanized and the stomach and intestines were retrieved and immediately stored in -18°C for later analysis by microscopy. For microscopy, the intestine was cut open, divided in 6 cm sections, placed on glass slide and examined with a stereomicroscope (SteReo Discovery V8, Carl Zeiss MicroImaging GmbH, Jena, Germany).

For HPLC, the plasma samples were mixed 1:1 v/v ratio with 10 % trichloroacetic acid (TCA) and centrifuged at 10,000 RPM for 10 min and the supernatant was retrieved for HPLC analysis. The HPLC analyses were carried out on a Dionex Ultimate 3000 pump, equipped with a Dionex Ultimate 3000 autosampler and a UV-VIS lamp. The samples were run isocratic at an absorbance wavelength of 243 nm at ambient temperature with mobile phase A (Milli-Q water with 0.1 % v/v trifluoracetic acid) and B (acetonitrile) at a A:B ratio 95:5 v/v with a Kinetex 5.0 μm C18 100 Å, 100 x 4.6 mm column (Phenomenex ApS, Denmark). Injection volume was 20 μL at a flow rate of 0.8 mL/min with a run time of 10 min per sample. All results were normalized after the individual dose and the individual weight of each rat. Non-compartmental analysis was applied to determine PK parameters of the paracetamol levels in plasma of each rat. AUC of plasma concentration versus time was calculated by the linear log trapezoidal method and confirmed by column statistics in GraphPad Prism version 7.00 (San Diego, CA, USA). C_{max} and T_{max} were determined using the PK profiles. Furthermore, relative bioavailability (F_{Rel}) was calculated by the following equation:

$$F_{Rel} = 100 \; x \; \frac{AUC_{PCL} \; x \; D_{control}}{AUG_{control} x D_{PCL}} \qquad (1)$$

where AUC_{PCL} is the $AUC_{0-360 \; min}$ of each individual rat dosed with the PCL microcontainer formulation and $AUC_{control}$ is the $AUC_{0-360 \; min}$ of

each individual rat dosed with the control formulation, D_{PCL} is the dose of paracetamol in the PCL microcontainer formulation given to each rat, and $D_{control}$ is the dose of control formulation for each rat.

The results are shown as mean ± standard error of the mean (SEM). Statistical significance was tested with an unpaired Student's t-test with Welch's correction if variance was significantly different (tested by F-test) with a p-value < 0.05 considered as significant.

Conclusions

The development of oral drug delivery systems for administering drugs in a safe and effective manner in the GIT is of high importance. This study demonstrates the successful fabrication of biodegradable microcontainers using hot punching. The novel process based on assembly of compression molded polymer films avoids the need of solvents or expensive batch processes. In a single processing step, simultaneous patterning of the PCL and thermal bonding to the underlying PVA substrate is achieved, resulting in replication of large arrays of microcontainers on a water soluble substrate. A modified powder embossing technique has been developed to uniformly load drug into the reservoirs of the microcontainers. This method allows a fast and precise drug loading without the need for alignment of microcontainers to a shadow mask. The fabrication procedure lends itself to continuous manufacturing processes such as in a roll-to-roll (R2R) configuration and is potentially applicable for other polymers and drug formulations. A pH-sensitive lid of Eudragit® S100 was applied as a coating on the microcontainers leading to a more targeted system where the drug does not degrade in the harsh gastric conditions before reaching the intestine. *In vitro* studies confirmed that the drug release is prevented from the acidic environments in the stomach. Finally, this work includes the first *in vivo* studies of drug absorption from biodegradable microcontainers indicating a higher bioavailability compared to conventional dosage forms, which is an important step towards real life applications of microfabricated drug delivery systems.

Conflicts of interest

There are no conflicts to declare.

Authors' contribution statement

Z.A: Conceptualization, Data Curation, Formal analysis, Project administration, Investigation, Methodology, Visualization, Writing – original draft, Writing – review and editing. *S.A*: Data curation, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review and editing. *M.J*: Investigation. *C.M*: Investigation, Formal analysis, Writing – Original draft. *L.V*: Conceptualization. *L.N.*: Formal analysis, Supervision, Writing – review and editing. *C.G*: Data curation, Investigation, Writing – review and editing. *R.P*: Writing – review and editing, Supervision. *A.M*: Writing – review and editing, Resources, Supervision. *A.B*: Funding acquisition, Project administration, Resources, Supervision, Writing – review and editing. *S.K*: Conceptualization, Supervision, Formal analysis, Funding acquisition, Investigation, Resources, Writing – original draft, Writing – review and editing

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