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Toward Combination Therapy

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Sequential Drug Release Achieved with Dual-Compartment Microcontainers: Toward Combination Therapy

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Combination drug therapy is commonly used to treat cancer, diabetes, cardiovascular conditions, and infections. However, these therapies face challenges associated with patient compliance and toxicity. Over the past decades, microdevices have emerged as a promising candidate for oral delivery allowing for targeted drug delivery with a tunable drug release. In the present work, engineered and monodisperse dual-compartment microdevices are developed to achieve a physical separation of two drugs followed by a sequential release in the gastrointestinal tract. As proof-of-concept, the compartments are sealed with two pH-sensitive polymers of different thicknesses to control the sequential release of propranolol and furosemide. In vitro release studies and in vivo absorption studies in rats confirm a sequential drug release from the two compartments. Unlike other proposed approaches, it is highly advantageous that the drugs can be loaded directly as powders, and that their release can be tuned via optimized coatings to achieve the desired release and absorption profiles. Conclusively, this study lays a strong foundation for the future use of microdevices to enable co-delivery of drugs followed by a sequential release in close proximity in the gastrointestinal tract.

1. Introduction

Combination drug therapy is commonly used in disease treatment, including in the treatment of cancer, cardiovascular conditions, HIV, and drug-resistance infections.[1–3] For example, a short term combination therapy, such as rifampicin and isoniazid, is one of the preferred approaches to treat tuberculosis and avoid drug resistance and transmission of the disease.[4] Many different oral drug delivery strategies have been investigated for co-delivery of multiple drugs in one dosage form, such as tablets, liposomes, 3D printed macrodevices, micro/nanoparticles, micelles, polymersomes, and dendrimers.[1,5–11] Furthermore, co-delivery and sequential release of different pharmaceutical compounds from orally administered microcapsules has shown promising in vitro and in vivo results as an efficient strategy for the treatment of renal fibrosis.[12]

Since 2012, polymeric microdevices with an inner reservoir for loading of drugs have attracted increasing attention for oral drug delivery.[15] The advantages of such devices, over traditional micro- and nanoparticle-based drug delivery systems, are many. First, versatile and scalable fabrication processes make it possible to realize monodisperse devices that offer protection of loaded drugs through the harsh gastric environment.[16–19] Second, the structure of the devices allows for a unidirectional release in the intestine, which entails an increased local concentration of drug molecules close to the intestinal wall. This can in turn result in higher oral bioavailability when comparing to omnidirectional formulations such as traditional tablets or particles.[15–18,20] Finally, reservoir-based microdevices can prolong the gastrointestinal (GI) residence time due to mucus attachment.[18,21–24] Altogether, these microdevices have an enormous potential for increasing the absorption of drugs, but so far, they have mainly been used to deliver a single drug at a time.[18,23–27]

In a few cases, reservoir-based microdevices have been prepared for oral delivery of multiple drugs, however, without achieving sequential release, but merely simultaneous release of pharmaceutical compounds for therapeutic applications such as drug-resistant infections, diabetes, and cancer.[28–30] In one case, the sequential release was obtained from a flat microdevice with
Figure 1. A) Schematic of the workflow to load, coat, and fill DCMCs into capsules. Notice the two different pH-sensitive polymers, Eudragit S-100 and Eudragit L-100 (dissolving above pH 7 and pH 6, respectively), applied on the inner and outer compartment. These two coatings were applied to delay the drug release from the inner compartment based on both the pH-value and the thickness of the coating. B) Process flow overview of the capsule dissolution and C) sequential release of the drugs in the GI tract.

A few engineered millimeter-sized 3D printed devices have been developed for simultaneous\(^\text{8,12,13}\) and sequential drug release\(^\text{34–37}\). For a couple of these devices, evaluation was performed both in vitro and in vivo.\(^\text{36,37}\) Genina et al. developed a macro-device where high-dose drug filaments, produced by hot melt extrusion, were loaded into two physically separated compartments. One of the compartments was sealed with a water-soluble cap of polyvinyl alcohol to obtain a sequential release. Despite promising in vitro results, the observed sequential release could not be reproduced in vivo after oral dosing to rats.\(^\text{16}\)

The aim of the present work was to: i) develop and fabricate a microdevice for sequential drug release in close proximity in the gut; and ii) prove this concept both in vitro and in vivo. Based on a long series of empirical results outlining the advantages of traditional microdevices (drug protection, unidirectional release, mucus embedment), we developed a dual-compartment micro-container (DCMC) for drug delivery with possible future applications in the field of combination therapy (Figure 1). Compared to previous microdevices, our device design features two separate compartments, a cylindrical inner and a concentric outer compartment, with different diameters and heights (Figure 1). This enables individual filling and sealing of the compartments using standard materials (e.g., formulations in powder form). The DCMCs were fabricated in a biocompatible photopolymer (SU-8),\(^\text{38}\) and methods for drug loading and polymer coating were established and optimized prior to in vitro and in vivo testing.

With the development of DCMCs, we introduce a novel design and combination of different approaches to fabricate microdevices and load them with drugs, in order to achieve sequential release from separate compartments in the same device. Previous studies have focused on attaining sequential delivery by modifying how the drugs are loaded into the device (e.g., loading in hydrogels). In contrast, we load the drugs in its pure powder form and apply two different polymeric coating layers. The ability to load powder directly into the device results in multiple advantages, such as a larger loading capacity and a broader applicability (i.e., can easily be adapted to work with different drugs or polymeric coatings).

To evaluate the potential of the developed device in vitro and in vivo, the DCMCs were filled with two model drugs, furosemide and propranolol. The two drugs are commonly used as a combination therapy of hypertensive disorders and are often quantitatively analyzed together.\(^\text{39–41}\) To secure an effective sequential release, the inner compartment was sealed with Eudragit S100 (dissolving above pH 7), while both the inner and outer compartment was coated with Eudragit L100 (dissolving above pH 6). Due to different threshold pH-values, Eudragit L100 will dissolve faster than Eudragit S100 at the same pH value. The developed
DCMCs were investigated in vitro and in vivo to assess the release profiles and their effect on drug absorption following oral dosing to rats.

2. Results and Discussion

2.1. Design and Fabrication of DCMCs

The DCMCs were designed to allow for a physical separation of two drugs and provide a unidirectional release upon dissolution of the enteric coatings covering the compartments. The DCMCs were fabricated in a biocompatible photopolymer (SU-8) by UV lithography, following an approach initially introduced for making microcontainers by Tao et al.\(^\text{[42]}\) and later modified and utilized extensively.\(^\text{[19,21,27,43,44]}\) In three consecutive lithography steps, the bottom, the outer sidewalls (terminating the outer compartment), and the inner compartment were defined by UV exposure and thermal curing before finally developing the microscale DCMCs (Figure 2A). By producing the DCMCs using UV lithography in SU-8, tight dimensional tolerances can be maintained on the fabricated structures. The structures were fabricated on a substrate with nine chips, each consisting of 324 DCMCs (Figure 2B). The two separate compartments in the DCMC were designed to have different diameters and heights (Figure 2C–E).
allowing for individual loading of two drugs into the same device. The possibility of having the drugs physically separated can limit unwanted drug–drug interactions and allow for a highly deterministic sequential release, which altogether can enhance the efficacy of combination drug therapies.[36]

Following characterization with optical microscopy and profilometry, the dimensions of the produced DCMCs were found to have negligible variations (Figure 2D,E). As the DCMCs were used for co-release of drugs, the available volumes in both compartments were important in terms of estimating the drug-loading capabilities. The volume of the inner and outer compartment was calculated to be 7.2 ± 0.3 and 5.0 ± 0.6 nL, respectively, and the taper angles of sidewalls interfacing the compartments showed minor variations (θ = 83 ± 1°).

2.2. Enteric Coating and Drug Loading of DCMCs

To obtain successful sequential delivery in the GI tract, there are two main strategies to employ: either time or pH-dependent delivery. In this study, we opted for a combination of the two strategies by sealing the two compartments with different thicknesses of two pH-sensitive polymers, dissolving at slightly different pH values. Since the pH in the proximal part of the small intestine of rats is 7.5,[45] Eudragit S100 (dissolving above pH 7) was used to seal the inner compartment and Eudragit L100 (dissolving above pH 6) for subsequent coating of both compartments (Figure 3A), which resulted in a double layer covering the inner compartment. Thus, the polymeric coatings dissolve with different rates immediately upon entry into the intestinal environment, which, together with the varying coating thickness, allows for a sequential release from the two compartments in a localized manner.

A scanning electron microscope (SEM) examination of the sealed DCMCs confirmed that the applied coatings were uniform and that the cavities were completely covered (Figure 3B–D). Due to the different chemical properties of the polymers, the drug in the inner compartment can be expected to be released later and at a higher pH value than the drug in the outer compartment, resulting in a sequential release in the GI tract. This strategy stands out compared to previous studies, where the focus has mainly been on achieving sequential delivery by relying solely on time-dependent delivery, for example, by encapsulating the drugs into hydrogels,[31] or applying a water soluble cap polymer to delay the release.[46]

The compartments were loaded using shadow masks. For loading of the inner compartment, a nickel shadow mask (Figure 3E) was made by electroplating from a seed layer on a silicon substrate with predefined SU-8 pillars (Figure 3F). For loading of the outer compartment, a polydimethylsiloxane (PDMS) mask[46] was applied to cover the spaces between the DCMCs (Figure 3C). The SEM micrographs show that a precise and efficient loading was achieved for both the inner and outer compartment (Figure 3C,G, respectively). Compared to previously developed sequential release devices,[31,35,36] it is an essential advantage that the drugs can be loaded directly into the DCMCs without any additional modifications. Previously, it has been necessary to load a hydrogel matrix or extrude into filaments[31,35,36] to obtain a time-dependent release of two drugs. It is far less labor-intensive when a powder can be loaded directly into a microdevice, and the dose in a single device can be remarkably larger. Per DCMC, a loading of 3.4 ± 0.3 μg furosemide and 3.3 ± 0.2 μg propranolol could be obtained for the inner and outer compartment, respectively (calculated based on the amount of drug loaded per chip with 32 DCMCs) (Figure 3H). As one example, there is a factor 100 increase in dose compared to a similarly sized device (200 μm diameter), capable of delivering 27 ng of three different drugs since the drugs were loaded into hydrogels to achieve a time-dependent drug release.[31] A slightly larger loading capacity was observed when the two drugs were loaded oppositely (i.e., propranolol in the inner compartment and furosemide in the outer compartment (Figure 3I). This is ascribed to the slightly larger volume of the inner compartment (7.2 ± 0.3 nL vs 5.0 ± 0.6 nL) together with differences in the compaction degree for the two drugs.

2.3. In Vitro Release Studies

To assess if the DCMCs could enable a successful sequential release of two drugs in vitro, the release of furosemide and propranolol was measured using a μDiss Profiler. Here, in situ fiber optic probes continuously tracked the released amount of the two drugs over time (Figure 4A), thus providing a real-time picture of the individual release profiles (Figure 4B,C). The in vitro release was performed in phosphate buffered saline (PBS) (pH 7.5), and in a two-step release model simulating the gastric and intestinal fluids in fasted rats (30 min in fasted state simulated gastric fluid (FaSSGF) pH 2.4 followed by 150 min in fasted state simulate intestinal fluid (FaSSIF) pH 7.5).[45]

Both in PBS and in simulated GI fluids, the DCMCs enabled a sequential release of propranolol (outer compartment) and then furosemide (inner compartment) with a time gap of 30 min (Figure 4B). Furthermore, the enteric coating on the DCMCs provided the protection associated with the pH-sensitive coating used for the inner compartment during the gastric step, resulting in no release of furosemide during the first 30 min (Figure 4B). An early release of propranolol could be observed from the outer compartment toward the last part of the gastric step (Figure 4B). A similar premature release of the drug (10–20%) after extended exposure to a medium with low pH has been observed in previous studies.[17,18,44] In the present case, it is hypothesized that the early release of propranolol from the outer compartment, which was not observed for furosemide, was caused by a combination of two things: 1) the higher water solubility of propranolol compared to furosemide; and 2) the thinner coating layer on the outer compartment compared to the inner compartment (40 vs 60 passages, respectively). To confirm that the premature release was not simply the result of a dissolved coating layer, the DCMCs were inspected using SEM after the gastric step (Figure 4B). Small cracks could be observed on the surface of the enteric coating. It is plausible that the micro-cracks are merely associated with the low chamber pressure (30–50 Pa) during SEM inspection, as it has previously been observed.[47]

For inversely loaded DCMCs, a different type of sequential release could be observed for the two drugs, where furosemide (outer compartment) and propranolol (inner compartment) were released at the same time, but with different rates (Figure 4C). The reason for the more pronounced time gap between the release of propranolol and furosemide in the loaded (not inversely)
Figure 3. Drug loading and polymer lid coating. A) Schematic illustrating the overall concept of the developed drug loading and polymer coating method: A nickel shadow mask was applied onto the empty DCMCs covering everything but the inner compartment. The inner compartment was then loaded, the mask was removed and the DCMCs were coated with Eudragit S100. Then, a PDMS mask was applied between the DCMCs in order to load the outer compartment before the mask was removed and the entire DCMC was coated with Eudragit L100. B) SEM image of a DCMC loaded with drug in the inner compartment and coated with Eudragit S100 (100 μm scale bar). C) SEM image of a DCMC with a drug-loaded and coated inner compartment, and a loaded outer compartment with a PDMS mask covering the space between the containers (100 μm scale bar). D) SEM image of the final DCMC, ready to be loaded into a capsule for oral dosing (100 μm scale bar). E) Picture of the nickel shadow mask after electroplating, laser cutting, and cleaning. The outer dimension of the mask is 12.8 × 12.8 mm² and the thickness is ≈200 μm. F) VSI data on the SU-8 pillars after development. The height is ≈250 μm and the diameter at the top surface is 330 ± 2.2 μm. G) SEM images showing the nickel shadow mask aligned to the inner compartment (top) and the furosemide loaded compartments after mask removal (bottom) (300 μm scale bars). H) Bar plots illustrating the drug loading capacity of DCMCs loaded with furosemide (inner compartment) and propranolol (outer compartment) dosed to each rat in the animal study (mean ± SD, n = 10). I) Bar plots illustrating the drug loading capacity of inversely loaded DCMCs loaded with furosemide (outer compartment) and propranolol (inner compartment) dosed to each rat in the animal study (mean ± SD, n = 10).
Figure 4. In vitro sequential release studies. A) The μDiss Profiler setup applied to study the sequential release in vitro. Six individual fiber optic probes allow for simultaneous measurements of up to six replicates at a time. A chip with DCMCs was attached to a cylindrical magnet, and the sequential release was measured as the concentration of the two drugs in solution over time. B) In vitro release of propranolol (outer compartment) and furosemide (inner compartment) from DCMCs in PBS (left) and in gastric and intestinal media (right) over time. The SEM image illustrates that the coatings appear intact after the gastric step, even if a release of propranolol could be observed from the outer compartment during the gastric step (300 μm scale bar). C) In vitro release of propranolol (inner compartment) and furosemide (outer compartment) from inversely loaded DCMCs in PBS (left) and in gastric and intestinal media over time (right). The SEM image illustrates that both compartments in the DCMCs were completely empty at the end of the release experiment (300 μm scale bar). All data represents mean ± SD. For studies in PBS n = 4, and for studies in media n = 3.

DCMCs can be attributed to the poor solubility of furosemide in the aqueous medium (0.01825 mg mL⁻¹) compared to propranolol (50 mg mL⁻¹). Despite the differences in aqueous solubility, both drugs were completely released from the DCMCs at the end of the in vitro experiments (Figure 4C). Differences in the aqueous solubility of the drugs co-loaded into a device for sequential release have previously affected the in vitro release profiles. The authors reported 80% less in vitro release of the poorly soluble rifampicin compared to isoniazid, from the compartment designed to delay the release. Furthermore, the release medium also affects the sequential release. In the present study, a faster furosemide release was observed for both versions of the DCMCs when the study was performed in biorelevant media compared to PBS. Propranolol, on the other hand, was less affected. This effect on furosemide release has previously been observed in the presence of bile salts and phospholipids.

Consequently, the presented in vitro data revealed that it is indeed possible to produce a sequential co-release of furosemide and propranolol by the developed DCMCs. The Eudragit coatings can be used to delay the release of the drug loaded into the inner compartment, and, thereby, work as an effective lid for the produced microdevices.

2.4. In Vivo Pharmacokinetic Studies

The in vivo studies aimed to investigate if sequential release and absorption could be quantified and verified for drugs delivered in the inner and outer compartment of the DCMCs, following...
oral administration to rats (Figure 5A). To load the DCMCs into gelatin capsules suitable for oral dosing, the DCMCs were detached from the silicon carrier substrate. SEM analysis of the detached DCMCs ensured that the devices were intact and that the coating layers were preserved after detachment (Figure 5B,C).

Three groups of rats were dosed with gelatin capsules containing: 1) DCMCs (furosemide in the inner and propranolol in the outer compartment), 2) inversely loaded DCMCs (propranolol in the inner and furosemide in the outer compartment), or 3) both drugs as free powder. The two DCMC groups allowed us to assess two complementary aspects: i) the absorption of the same drug delivered in the inner and outer compartment of the DCMCs; and ii) the absorption of two different drugs delivered in the inner and outer compartment of the DCMCs. The first aspect is important to ensure that a possible sequential absorption of the drugs in the two compartments is not simply due to drug-specific properties affecting absorption.

Focusing on the first of the two aspects (i.e., absorption of the same drug from the two compartments), the plasma concentration profiles for furosemide, either dosed in the inner or outer compartment of DCMCs or as free powder in gelatin capsules with oral gavage to rats (mean ± SE, n = 4–5). F) AUC_{0-120 min} of furosemide after dosing in the inner or outer compartment of DCMCs or as powder in gelatin capsules (mean ± SE, n = 4–5). * indicates significant difference between the groups. G) Optical microscopy images of empty DCMCs embedded into the mucus and intestinal content in the ileum after dosing to rats (300 μm scale bar).
drug is delivered in the outer compartment of the DCMCs compared to in the inner compartment (Figure 5D). This rank-order corresponds well with the in vitro observations (Figure 4B,C), where the release occurred first from the outer compartment followed by the inner compartment. The rank-order provided by the plasma concentration profiles of furosemide is further supported by the area under the curve for the first 120 min (AUC_{0-120 min}) for furosemide (Figure 5F). In this case the AUC_{0-120 min} was found to be lower when dosed in the inner compartment (5.3 ± 2.5 μg min mL^{-1}) than when loaded in the outer compartment (17.7 ± 3.0 μg min mL^{-1}) and significantly higher when dosed as free powder (24.8 ± 5.4 μg min mL^{-1}). Thus, the release and absorption of furosemide during the first 120 min followed the rank-order: inner compartment < outer compartment < free powder. This trend supports the conclusion that it is possible to achieve a delayed release by loading the drug in the inner compartment compared to the outer one, which again happens later than when the powder is dosed freely in a gelatin capsule.

For propranolol, a similar trend was observed from the plasma concentration profiles, where a slightly faster absorption of propranolol occurred after dosing as free powder compared to delivery in DCMCs (Figure 5E). As seen for furosemide, this was further supported by differences in the AUC_{0-120 min}. Propranolol dosed in the outer compartment of DCMCs resulted in an AUC_{0-120 min} of 0.44 ± 0.17 μg min mL^{-1}, which was found to be lower than the corresponding value from the powder group (1.11 ± 0.74 μg min mL^{-1}). Propranolol, could not be measured after dosed in the inner compartment. One possibility is that the propranolol was released slower from the inner compartment, resulting in a lower plasma concentration and, thus, lowers detection on the LC-mass spectrometry (MS).

Additionally, imaging of intestinal sections from two rats 2.5 h after dosing, revealed the presence of empty, but otherwise intact, DCMCs in the ileum (Figure 5G). These observations confirm that the DCMGs were structurally undamaged and non-digested as previously observed,\(^\text{[51]}\) and that the enteric coating was completely dissolved and the drugs successfully released from both compartments.

Besides clear differences in the absorption of furosemide during the first 3 h, the observed values of the time (T_{max}) when the maximum plasma concentration (C_{max}) is reached (Table 1) were similar, whether furosemide was released from the inner compartment (4.0 ± 0.5 h) or the outer compartment (4.2 ± 0.8 h). In contrast, the T_{max} observed for the free furosemide powder appeared earlier (2.2 ± 1.6 h), which is in accordance with literature values reported after oral administration of free furosemide powder to rats (0.8–2 h).\(^\text{[52,53]}\)

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<th>DCMCs</th>
<th>Inverse DCMCs</th>
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<td>Furosemide</td>
<td>Propranolol</td>
<td>Furosemide</td>
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<tr>
<td>T_{max} [h]</td>
<td>4.0 ± 0.5</td>
<td>1.5 ± 0.2</td>
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<td>C_{max} [μg mL^{-1}]</td>
<td>0.4 ± &lt;0.1</td>
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Based on the absorption of the two different drugs, it is observed that propranolol dosed in the outer compartment resulted in an earlier T_{max} than furosemide dosed in the inner compartment (1.5 ± 0.2 h and 4.0 ± 0.5 h, respectively). In contrast, propranolol and furosemide dosed as a free powder resulted in more similar T_{max} values (1.5 ± 0.6 h and 2.2 ± 1.6 h, respectively) (Table 1). This trend suggests that the DCMCs can be applied to achieve a sequential release of two drugs in vivo. However, when comparing the absorption of the two drugs from the outer compartment, propranolol presents an earlier T_{max} (1.5 ± 0.2 h) than furosemide (4.2 ± 0.8 h). This observation suggests that the release and absorption not only depends on device characteristics, but also relies on drug-specific properties.

In general, the C_{max} values for propranolol were found to be remarkably lower than for furosemide (Table 1), which we assigned to be due to the extensive first-pass metabolism of propranolol in the liver.\(^\text{[90]}\)

### 3. Conclusion

We have developed microdevices that hold promise for potential future use in combination drug therapy. Compared to previous work in this field, we demonstrate: a) sequential release secured by selective coatings; b) increase in drug loading capacity in powder form; and c) in vivo verification of sequential release of orally administered drugs.

DCMCs show promising potential for co-delivery and sequential release of drugs from two separate compartments. We have demonstrated that DCMCs can be successfully designed and reproducibly fabricated in the biocompatible photopolymer SU-8, and that it is possible to use them for physical separation and sequential release of two drugs from one single microdevice in vitro. Additionally, we showed that the drugs can be successfully loaded in their powder form, while modulating the release profile by pH sensitive polymers. The loading of the drugs in their powder form, compared to loading in for example hydrogels, allows for a larger degree of flexibility and enables delivery of larger drug doses in each device. In vivo, we showed that the release and absorption from the two compartments can be controlled to achieve different pharmacokinetic profiles. However, additional studies with different drugs will be required to fully understand how drug-specific properties influence the sequential release. For example sequential release of permeation enhancers and peptides or proteins will be interesting to study in the future.

This novel microdevice inherit all the benefits associated with microdevices for oral drug delivery (gastric protection, mucosal adhesion, unidirectional delivery etc.) while paving the way for next-generation combination drug therapy. The developed DCMCs

Table 1. Pharmacokinetic parameters of furosemide and propranolol following oral administration to rats after dosing in the inner and outer compartment of the DCMCs or as a powder mixture in gelatin capsules (mean ± SE, n = 4–5).
can be applied to deliver two pharmaceutical compounds to the GI tract in physically separate compartments, and thereafter provide a highly localized sequential release in the desired order. This can be beneficial for multiple co-delivery applications, for example for delivery of therapeutic peptides or proteins, where permeation enhancers are needed to be released first to modify the epithelial membrane and, thereby, facilitate absorption.

4. Experimental Section

Materials: Furosemide (≥98% purity) was purchased from Fagron NV (Rotterdam, Netherlands) and propranolol (99% purity) was obtained from Acros Organics (Geel, Belgium). Eudragit S100 and L100 were acquired from Evonik Industries (Darmstadt, Germany) and iso-propanol, acetonitrile, and methanol were bought from VWR International (Radnor, PA, USA). FaSSIF, FeSSIF, FaSSGF powder was from Biorelevant.com (London, United Kingdom). Dibutyl sebacate, sodium hydroxide, sodium chloride, sodium acetate, as well as PBS tablets, potassium phosphate monobasic, phosphoric acid, and acetic acid were all acquired from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid (32%) was purchased from Merck Darmstadt, Germany. The enzyme b-Glucuronidase/Arylsulfatase (≥100 000 Units mL\(^{-1}\)) was from Roche Diagnostics (Basel, Switzerland). Sygard 184 silicone kit was from Dow Chemical (Midland, MI, USA). Ultrapure water used throughout the study was obtained from a Q-POD dispensor from Merk Millipore (Burlington, MA, USA). The photoresist SU-8 was used for fabrication of DCMCs and the scaffold used for electroplating the shadow mask. Formulations with two different viscosities were used (i.e., SU-8 2035 and 2075) and the cross-linked structures were developed in m-Dev 600. Resist and developer were purchased from micro resist technology GmbH (Berlin, Germany). Single-side polished ø100 mm Si substrates with a thickness of 525 μm were acquired from Topsis GlobalWafers A/S (Frederiksdun, Denmark).

Design and Fabrication of DCMCs: The DCMCs were fabricated in the photopolymer SU-8 and produced in a three-step UV lithography process used for defining the bottom, the outer sidewalls, and the inner compartment in said order. Each step was composed of spin coating (RC80 manual spin coat, Süss Microtec, Germany), a soft bake (SB) for evaporation of solvent, UV exposure, with an illumination wavelength of 365 nm, carried out using a maskless aligner (MLA100 Tabloptable Maskless Aligner, Heidelberg Instruments, Germany) and finally a post exposure bake (PEB) to facilitate crosslinking of the exposed SU-8. All SBs and PEBs were conducted at 50 °C using a ramping rate of 2 °C min\(^{-1}\). For defining the 35 μm thick bottom layer SU-8 2035 was initially spin coated and UV-cured (SB time = 2 h, UV dose = 250 mJ (cm\(^{-2}\))\(^{-1}\), PEB time = 6 h). Subsequently, ≈130 μm thick SU-8 2075 layer was spin coated and cross-linked (SB time = 8 h, UV dose = 350 mJ (cm\(^{-2}\))\(^{-1}\), PEB time = 8 h) to form the outer sidewalls of the DCMCs. Finally, ≈85 μm thick SU-8 2075 layer was spin coated. The final UV exposure was aligned to the outer sidewalls and the full thickness (i.e., all three layers) was exposed to obtain the cylindrical inner compartment (SB time = 6 h, UV dose = 500 mJ (cm\(^{-2}\))\(^{-1}\), PEB time = 10 h). The final step in the production of the DCMCs was the development, which was performed in two dedicated baths of m-Dev 600 using an immersion time of 20 min in each bath. The developed DCMCs were flushed in copious amounts of isopropanol and left to dry for 2 h. Single-side polished ø100 mm Si carrier substrates were used during the fabrication of the DCMCs. A total of four substrates, each with nine chips containing 324 containers arranged in a quadractic 18 × 18 matrix, were produced. The individual chips were diced out (Automatic Dicing Saw DAD 321, DISCO, Japan) prior to the manual drug loading and lid coating. An anti-adhesion layer, composed of 5 nm Ti and 20 nm Au deposited by electron-beam evaporation (Temescal FC-2000, Ferrotec Corporation, USA), was added underneath DCMCs aimed at in vivo experiments. The presence of the anti-adhesion layer allowed for harvesting the drug-loaded and coated DCMCs without interfering bulk damage to the structures.

Topography Analysis: The topography analysis was conducted using bright-field optical microscopy (Nikon Eclipse L200, Nikon Metrology, Japan) for horizontal measurements and vertical scanning interferometry (VSI; Plu Neox 3D Optical Profiler, SensoFar Metrology, Spain) for vertical measurements and evaluation of the sidewall taper angle. The inner and outer diameters of the inner compartment as well as the outer SEM images were measured in the center of n = 12 chips. The bottom thickness and the inner heights of the inner and outer compartments were extracted from VSI data from n = 20 measurements. The volumes of both compartments were calculated from the topography data, where the taper angles of sidewalls interfacing the compartments were extracted from cross-sectional profiles generated from the VSI data. The optical profiling data was analyzed using the modular freeware program Gywiddion (http://gywiddion.net/).

Drug Loading and Polymer Spray Coating: The loading was carried out manually by pressing the powder into the DCMCs using a brush, as previously described.[18,54] First, everything except the inner compartment was covered with a nickel shadow mask to ensure selective loading into this compartment. After loading, the mask was manually detached and excess drug powder was removed using an air gun. After loading of the inner compartment, a lid of Eudragit S100 was deposited over the DCMCs, as previously described.[44] Briefly, an ultrasonic spray coating system (ExactaCoat system, Sono-Tek, Milton, NY, USA) equipped with an 120 kHz Acoustic nozzle was used to spray coat an isopropanol solution with 1% (w/v) Eudragit S100 and 0.5% (v/v) of the liquid plasticizer dibutyl sebacate. During spray coating, the generator power was kept at 2.2 W and the pump rate was 0.1 mL min\(^{-1}\). The speed of the nozzle was maintained at 10 mm s\(^{-1}\) and the shaping air pressure was set to 0.02 bar, keeping a distance between the tip and the sample of 4 cm. Each chip was coated with ten loops of two alternating spray paths having an offset of 2 mm, which resulted in a total of 20 passages. After loading and coating of the inner compartment, the outer cavity was loaded in a similar manner. However, instead of the nickel mask, a PDMS mask was applied to cover the spaces between the DCMCs as described in literature.[44] After the second loading step, the DCMCs were coated with Eudragit L100 following the same process as for the first Eudragit S100 lid by spray coating an iso-propanol solution with 1% (w/v) Eudragit L100, 0.5% (v/v) dibutyl sebacate, and 2% (v/v) MilliQ water. However, for Eudragit L100 each chip was coated with 20 loops, resulting in a total of 40 passages. The chips with DCMCs were weighed before and after both drug-loading steps in order to determine the loaded amount of drugs. Following the loading and coating processes, the chips were inspected using SEM (TM3030 Plus, Hitachi High-Technologies Europe, Krefeld, Germany) with an accelerating voltage of 15 kV.

In Vitro Release Studies: In vitro release of furosemide and propranolol from the coated DCMCs was measured using a µDisS Profiler (Pion Inc., Billerica, MA, USA), as previously described in literature.[18,54] The release was measured in PBS adjusted to pH 7.5, which was similar to the intestinal pH in fasted rats,[45] and in a biorelevant in vitro model consisting of two steps: 30 min in FaSSGF followed by release in FaSSIF until complete drug release from DCMCs. The biorelevant media were prepared as specified by the supplier, but the pH was adjusted to 2.4 and 7.5 for FaSSGF and FaSSIF, respectively. Furosemide and propranolol calibration curves were constructed by addition of different volumes from stock solutions of the respective drugs in PBS, FaSSGF, or FaSSIF (10 mL). In situ fiber-optic probes with a path length of 1 mm were applied and the absorbance was measured in the range of 360–370 nm for furosemide and 313–315 nm for propranolol. For the release experiments, the loaded and coated DCMC-chips were attached to cylindrical magnets, introduced into a sample vial and medium was added (10 mL). UV measurements were carried out every 10 s during 180 min in PBS or 30 min in FaSSGF followed by 150 min in FaSSIF. All studies were performed at 37 °C and with 100 rpm stirring with four replicates for the studies in PBS and three in the biorelevant media. SEM images were taken to inspect the chips after the gastric step and at the end of the release study with an accelerating voltage of 15 kV.

Animal Studies: All animal care and experimental studies were performed in accordance with the EC directive 2010/63/EU and with Danish law regulating experiments on animals under license.
(2015-15-0201-00553). The experimental protocols were approved by the Animal Welfare Committee, appointed by the Danish Ministry of Justice. Male Sprague-Dawley rats (weight of between 233 to 275 g on the day of the study) were purchased from Taconic Biosciences (Rensselaer, NY, USA), and housed in pairs to acclimate for a minimum period of 7 days with light/dark period of 12/12 h and under controlled environmental parameters (temperature: 20–22 °C, relative humidity: 55%). During this period, the animals had free access to standard pellets and water. On the day of the study, the rats were randomly divided into three groups (n = 6) and dosed with one gelatin capsule (Torpac size 9, Fairfield, NJ, USA) containing either DCMCs or a mixture of propranolol and furosemide powder. Before filling the DCMCs into capsules, SEM images were taken to inspect their integrity after detachment from the silicon chips with an accelerating voltage of 15 kV. Before the experiment, the rats were fasted for 2.5 h with ad libitum access to water, where after one capsule (1 mg furosemide and 1 mg propranolol) or an amount of DCMCs corresponding to 1 mg of each drug) was administered to each rat using a polyurethane feeding tube (Instech Laboratories Inc., Plymouth Meeting, USA). Blood (200 μL) was withdrawn from the lateral tail vein before dosing and after 0, 1, 2, 3 and 5 h. Immediately, the rats were opened and the small intestine was extracted. A light microscope (Zeiss Axio Scope.A1, Carl Zeiss, Göttingen, Germany) was used for localizing the DCMCs on the intestinal sections. The pharmacokinetics of furosemide in plasma of individual rats after oral administration were obtained by non-compartmental analysis. The pharmacokinetic parameters were based on described literature,[39] but optimized to the present setup. The mobile phase consisted of 0.02 M KH₂PO₄ (adjusted with acetic acid) and the enzyme B-glucuronidase/arylsulfatase (≥ 100 000 Units mL⁻¹) (5 μL) were added to plasma (20 μL). After vortexing, the tubes were incubated at 35 °C for 90 min. Then, cold methanol (64 μL, stored at −20 °C prior to use) was added to precipitate plasma proteins. The solution was vortexed, centrifuged (10 600 g for 10 min) and the supernatant was transferred to HPLC vials. A propranolol calibration curve was prepared by addition of different aliquots of propranolol stock solution in acetate buffer to blank plasma (20 μL). The standards were treated as described for the plasma samples but adding methanol (280 μL) for the precipitation of the proteins. The range of concentration was 0.003–0.041 μg mL⁻¹.

Quantitative Analysis of Furosemide in Plasma Samples by High-Performance Liquid Chromatography: The plasma samples were analyzed for the content of furosemide using a Shimadzu high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan), which consisted of a CBM-20A system controller, SIL-20ACHT auto sampler, LC20AT pump, DGU-20A5R degassing unit, CTO-20AC column oven, RID-20A refractive index detector, and SPD-20A photodiode array detector. All the HPLC parameters were based on described literature,[19] but optimized to the present setup. The mobile phase consisted of 0.02 M KH₂PO₄ and acetonitrile, mixed in a ratio of 75:25 (v/v) and pH adjusted to 3.9 using phosphoric acid. A Luna 5.0 μm C18 100 Å, 250 × 4.6 mm column (Phenomenex ApS, Værløse, Denmark) was used for the analyses placed upside down at ambient temperature. The auto sampler temperature was kept at 15 °C and a volume of 20 μL was injected. The flow rate was 1 mL min⁻¹ with a run time of 12 min for each sample. The absorbance was measured at 235 nm. Plasma samples were prepared for HPLC analysis by addition of cold methanol (100 μL, stored at −20 °C prior to use) to plasma (60 μL) in order to precipitate plasma proteins. Then, the mixtures were vortexed, centrifuged (10 600 g for 10 min) and the supernatant was transferred to HPLC vials. A furosemide calibration curve was collected by addition of different aliquots of furosemide stock solution in mobile phase to blank plasma (60 μL) and treated as described above for the plasma samples. The range of concentration was 0.036–1.6 μg mL⁻¹.

Quantitative Analysis of Propranolol in Plasma Samples by High-Performance Liquid Chromatography-Mass Spectrometry: Plasma samples and standards were analyzed for the amount of propranolol with a Shimadzu Nexera X2/Prominence HPLC (Shimadzu Europe, Duisburg, Germany) and ESI microOTOF-Q III (Bruker Daltonics, Bremen, Germany). HPLC-MS setup. The HPLC instrument consisted of a Nexera X2 SIL-30AC auto sampler, Nexera X2 LC-30AD solvent pumps, DGU-20A5R degasser, Prominence CTO-20AC column oven, and Prominence SPD-M20A diode array detector. The HPLC was performed by injection of the analyte (5 μL) on a Poroshell 120 SB-C8 column, 2.7 μm, 2.1 × 50 mm (Agilent, Santa Clara, CA, USA) followed by elution with a linear gradient of MeCN and 2.5 mM NH₄OH in water with 0.1% formic acid at a flow rate of 0.4 mL min⁻¹. The gradient program was as follows: 0–9 min, 0% MeCN concentration; 9–11 min, 100% MeCN concentration; 11–14 min, 0% MeCN concentration. Only the section of the chromatogram between 4.5 and 5.2 min was injected into the ion source, while the remaining run was diverted to waste. A calibration solution consisting of 2.5 mM NaOH, 2.25 mM formic acid in 90% i-ProH₂O/water was injected into the ion source between 4.5 and 4.6 min at a flow rate of 30 μL h⁻¹ for internal calibration of the spectra. MS analysis was performed in positive mode in the mass range 50–1500 m/z⁻¹ at a rate of 0.2 Hz. The HPLC-MS method was carried out using nitrogen to assist nebulization, stabilizing a nebulizer pressure of 0.7 bar. The capillary voltage of 4500 V was employed, and the end plate offset was set to −300 V. All mass spectra were analyzed with the software QuantAnalysis (Bruker Daltonics, Bremen, Germany) to generate extracted ion chromatograms for 260.16 ± 0.01 m/z⁻¹ and the resulting peaks were integrated. Plasma samples were exposed to an enzymatical hydrolysis treatment as previously described in literature.[31] For that purpose, 0.1 mM acetic buffer (5 μL) of pH 5 (adjusted with acetic acid) and the enzyme B-glucuronidase/arylsulfatase (≥ 100 000 Units mL⁻¹) (5 μL) were added to plasma (20 μL). After vortexing, the tubes were incubated at 35 °C for 90 min. Then, cold methanol (64 μL, stored at −20 °C prior to use) was added to precipitate plasma proteins. The solution was vortexed, centrifuged (10 600 g for 10 min) and the supernatant was transferred to HPLC vials. A propranolol calibration curve was prepared by addition of different aliquots of propranolol stock solution in acetate buffer to blank plasma (20 μL). The standards were treated as described for the plasma samples but adding methanol (280 μL) for the precipitation of the proteins. The range of concentration was 0.003–0.041 μg mL⁻¹.

Statistics: The obtained results are shown as the mean ± error, and it is stated in the figure captions whether standard deviation (SD) or standard error of the mean (SE) are shown. Where appropriate, statistical analysis was carried out in GraphPad Prism version 6.0 (GraphPad software, San Diego, CA, USA) using a Student’s t-test. P-values below 5% (p < 0.05) were considered statistical significant.

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Conflict of Interest
The authors declare no conflict of interest.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.