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Powder embossing method for selective loading of polymeric microcontainers with drug formulation

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Abstract

The present study introduces powder embossing as a novel method to enhance loading of polymeric microcontainers with drug. With current loading approaches, it is not possible to handle pure powder drug in a scalable, homogenous and reproducible manner. In this work, we demonstrate simultaneous loading of 625 microcontainers with powder formulation. This is achieved in a single step by aligning a shadow mask prepared by micro-milling to an array of microcontainers in order to limit drug deposition to the container cavities with diameters of 220 µm. A pressure of 8.9 MPa is applied by a bonding press and thereby the desired powder is embossed into the container cavities. Powder in the form of pure drug, lipid-based microparticles, and pure polymer was successfully loaded with minimal residues in between the microcontainers and with 100% loaded cavities demonstrating the versatility of the method. The current work is thus contributing to the loading of powder formulations into microscale drug delivery systems such as microcontainers in a facile and reproducible manner.

Keywords: Microcontainers, shadow mask, micro-milling, drug delivery systems, microtomography, oral drug delivery

1 Introduction

In recent years, microfabricated devices have been proposed as advanced drug delivery systems [1][2][3]. Microfabrication methods allow the definition of devices with well-defined geometry and size containing a precise amount of drug in each unit and enabling controlled release. In particular, microcontainers have been presented as promising new advanced oral drug delivery systems with the potential to significantly enhance the bioavailability of drugs[4][5][6]. These microcontainers consist of walls and a bottom defining a drug reservoir with a volume in the pL to nL range. In contrast to the traditional oral drug delivery systems such as tablets, microcontainers provide a larger surface to volume ratio. This, in some cases combined with the integration of mucoadhesive features, promotes attachment of the drug delivery systems to the intestinal mucosa and a unidirectional drug release due to a cavity open only on one side [7]. Due to their small dimensions, one of the major challenges is to load drug into the microcontainers. A suitable method has to avoid damaging the drug while achieving a homogeneous and reproducible loading.

In the past, various methods for drug loading into microcontainers have been proposed. Ainslie et al. proposed UV crosslinking of hydrogel matrices with drug. However the amount of drug that can be loaded with this approach is very restricted [8][3]. Alternatively, hot punching in a spin-coated drug-polymer film or supercritical impregnation of microcontainers filled with polymer by inkjet-printing were demonstrated [9][10]. In all these methods, solubility of the drug in the polymer matrix is required. Furthermore, the polymer matrix itself will occupy a considerable part of the container volume thereby reducing the amount of drug that can be loaded.

Typically, drugs are available as powder acquired from commercial suppliers or prepared by spray drying and it is relevant to develop a technique where pure powder drug can be loaded into the microcontainers.

In the existing powder filling method for polymeric microcontainers [11], the powder is manually deposited on the microcontainers and compacted with a spatula. The residual amount of drug between the containers is blown away with pressurized air. This method is not applicable for sticky powder such as spray-dried lipid-based microparticles. Moreover, this method provides irreproducible loading and results in considerable waste of drug due to the use of pressurized air both removing powder in-between but often also from the upper part of the container reservoir.

Here, we present an improved method for loading microcontainers with powder formulation. This is achieved by clamping a shadow mask between arrays of microcontainers followed by embossing of the desired powder formulation into the cavities of the microcontainers. The overall concept is illustrated in figure 1.

The shadow mask allows a more precise loading of powder formulations as manual distribution of powder and the use of the pressurized air can be avoided. For the fabrication of shadow masks, a large number of materials and methods have been suggested for other applications. Silicon (Si) shadow masks have been used for metal evaporation or local plasma polymerization[12][13]. A ferromagnetic Ni shadow mask was presented to allow the use of magnetic forces to provide clamping between substrate and stencil[14]. However, in both cases preparation of the shadow masks is both time consuming and requires various cleanroom processes. In this work, a micro-milled aluminum shadow mask is suggested with the purpose of embossing powder into the container cavities without leaving residues around the microcontainers. In order to explore the versatility of this method, spray-dried lipid-based micro-particles, drug in pure form and polymer are loaded into the containers.

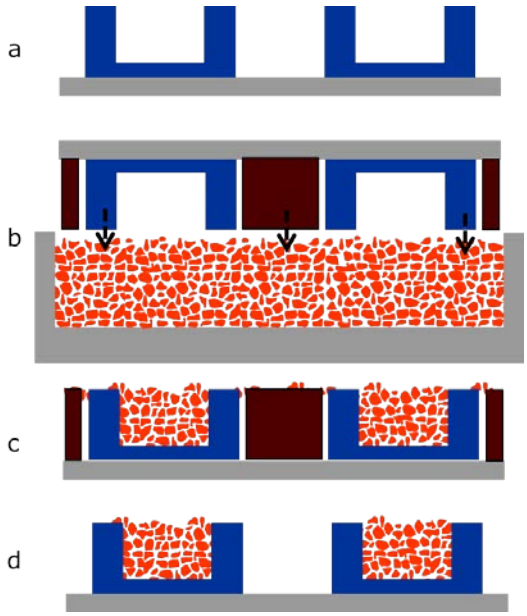

Figure 1 Illustration of the method for loading micro containers with powder formulation. (a) SU-8 microcontainers (blue) fabricated on Si carrier substrate (grey); (b) the shadow mask (brown) is clamped to the microcontainers. Powder formulation (red) is placed in a holder in order to have equal amount of powder in each loading step and pressure is applied (c) After the pressure is released (d) the shadow mask is removed and the containers are uniformly loaded with powder.

2 Materials and methods

2.1 SU-8 microcontainers

Silicon wafers (4-in. b100N n-type) were supplied by Okmetic (Vantaa, Finland). SU-8 2075, SU-8 2035 and SU-8 developer were purchased from Microresist Technology GmbH (Berlin, Germany). Cylindrical SU-8 microcontainers were fabricated on Si substrates with a similar method as described previously [11]. The microcontainers had an outer diameter of 300 µm and a height of 300 µm. The microcontainer reservoir had a diameter of 220 µm and a depth of 270 µm. Following fabrication, the wafer was cut into 12.8x12.8 mm^2 square chips (DISCO DAD 321, Automatic Dicing Saw). Each of these microcontainer chips contained 625 microcontainers arranged in an array of 25x25 with a center-to-center distance of 450 µm.

2.2 Micro-milling of shadow mask and alignment tool

The shadow mask was designed using solidworks and micro-milling procedures were generated using Cimatron. 300 µm and 1 mm endmill tools were used for fabrication of the mask through micro-milling in 300 µm thick aluminum sheets. The side length of the shadow mask was 15x15 mm^2. The holes of the shadow mask had a diameter of 380 µm and a center-to-center distance of 450 µm. A separate alignment tool for the shadow mask was fabricated for an easy alignment and clamping to the microcontainers as illustrated in figure 2.

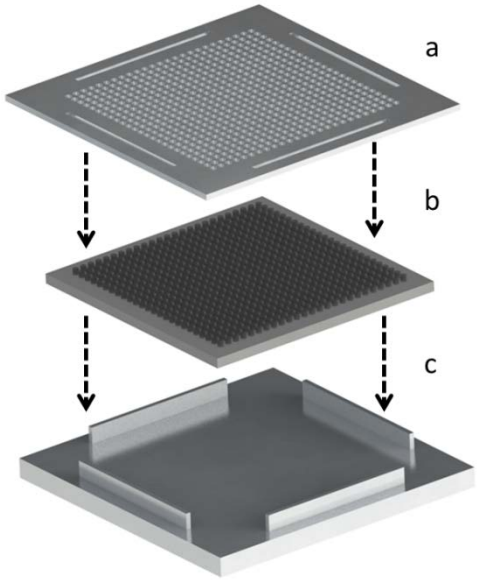

Figure 2 Illustration of the clamping of the shadow mask (a) onto the microcontainer chip with an array of 625 microcontainers (b). A separate bottom part (c) is designed to facilitate alignment of the shadow mask to the container chip.

2.3 Powder embossing for drug loading

The fabricated shadow mask was clamped to the array of microcontainers. For uniform transfer of the powder to the containers, the desired powder formulation was placed in a micro-milled recess with lateral dimensions of 15 mm^2 and a depth of 1 mm. By applying a pressure of 8.9 MPa (force 2 kN) with a bonding press (P/O/Weber), the powder was embossed inside the container cavities after which the pressure and shadow mask were gently removed. The remaining powder was reused for loading of following samples. Microcontainer chips were loaded with three different powder formulations: Furosemide (≥98% purity) was purchased from Fagron Nordic (Copenhagen, Denmark) as a model for pure drug powder loading; Polyvinylpyrrolidone (PVP) with an average mol wt 10,000 Da (K10) was purchased from Sigma-Aldrich (St. Louis) to evaluate loading of pure polymer powder; lipid-based microparticles in the form of empty cubosomes were prepared by spray drying with a similar method as described in an earlier study, here without addition of ovalbumin [15]. The investigations were carried out using ZEISS Supra 40 VP SEM. The samples were sputtered with Au before imaging to avoid charging of the non-conducting materials.

2.4 X-Ray microtomography

Sample chips as described in section 2.1 were analysed using a commercial X-ray microtomography versa system (Zeiss Xradia 410). The system has an X-ray source operated in reflection geometry, a working high voltage between 40 kV and 150 kV and a power up to 10W.

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 objectives. Each sample was imaged first with a low resolution using the Large Field Of View objective (19.68 µm pixels and a collection time of 2 hours, using 1601 projection Images to cover 360 degrees rotation) in order to observe the entire chip of 625 containers. Then, an area of interest was selected for further investigation with a higher resolution (3.02 µm pixels and a collection time of 5.5 hours with 3201 projection images covering 360 degree rotation) to properly investigate the loading of 25 microcontainers using the '4X' objective. Single containers were selected for thorough inspection with an even higher resolution (1.21 µm pixels with a collection time of 19 hours while rotating the sample 360 degrees in 3201 projection images). 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[16].

2.5 In vitro drug release study of furosemide from microcontainers

The *in vitro* release of furosemide from the microcontainers was tested using a µ-Diss profiler (pION INC, Woburn, MA, USA) in a similar set-up as described earlier (Nielsen et al., 2015, 2014). Experiments were carried out at 37^0C 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 microcontainer chips were attached to cylindrical magnetic stirring bars using carbon pads, placed in the bottom of sample vials, and covered with 10 mL of 100 mM phosphate buffer pH 6.5 for 20 h. The experiment was performed in 5 replicates (N=5).

3 Results and discussion

3.1 Alignment of shadow mask and powder embossing into microcontainers

Figure 3a-c shows that it was possible to achieve a perfect alignment and clamping of the containers to the shadow mask by placing a silicon chip with microcontainers on the alignment tool and then positioning the shadow mask on top of the containers. A successful loading is characterized by drug inside all container cavities. The powder filling required few seconds to be performed and resulted all of the 625 microcontainers in the array loaded in one single step corresponding to 100 % yield. This is shown for the three powder formulations with representative images in figure 3d-f. The powder granules were pressed into the containers and this confinement prevented them from falling out of the reservoir. Minimal residues in between the containers were observed. Despite the simplicity of the method, the SEM images show a uniform loading in each container. The average amount of micro-particles in the form of cubosomes was 1.7 ± 0.2 mg chips (n=5 chips), furosemide had an average weight of 1.5 ± 0.4 mg (n=5 chips), and PVP 1.3 ± 0.2 mg (n=5 chips). This corresponds to 2.7 µg/container, 2.5 µg/container and 2.1 µg/container, respectively.

3.2 Loading of particles with different morphology

X ray microtomography measurements have previously successfully been used to visualize the effect of loading procedures into microcontainers [10]. To visualize the effect of the process on the powder and to verify if the container reservoirs were completely filled, X-ray microtomography measurements were performed on the microcontainers loaded with the powder embossing method. As seen in the cross-sections reconstructed from X-ray microtomography measurements in figure 4a-c, all three powder formulations were successfully loaded inside the containers using the powder embossing method. Due to different particle morphology and size of the three powder types their distribution in the containers was different. The small lipid-based microparticles were compacted inside the containers without any inclusion of air. Furosemide was also densely packed inside the containers but some air was observed. Containers loaded with PVP displayed the presence of more spacing between the powder granules compared to the two previous materials. However, the loading with PVP was much more uniform compared to earlier work where containers were loaded without embossing [10]. These images demonstrate the variety of powders that can be loaded with this method.

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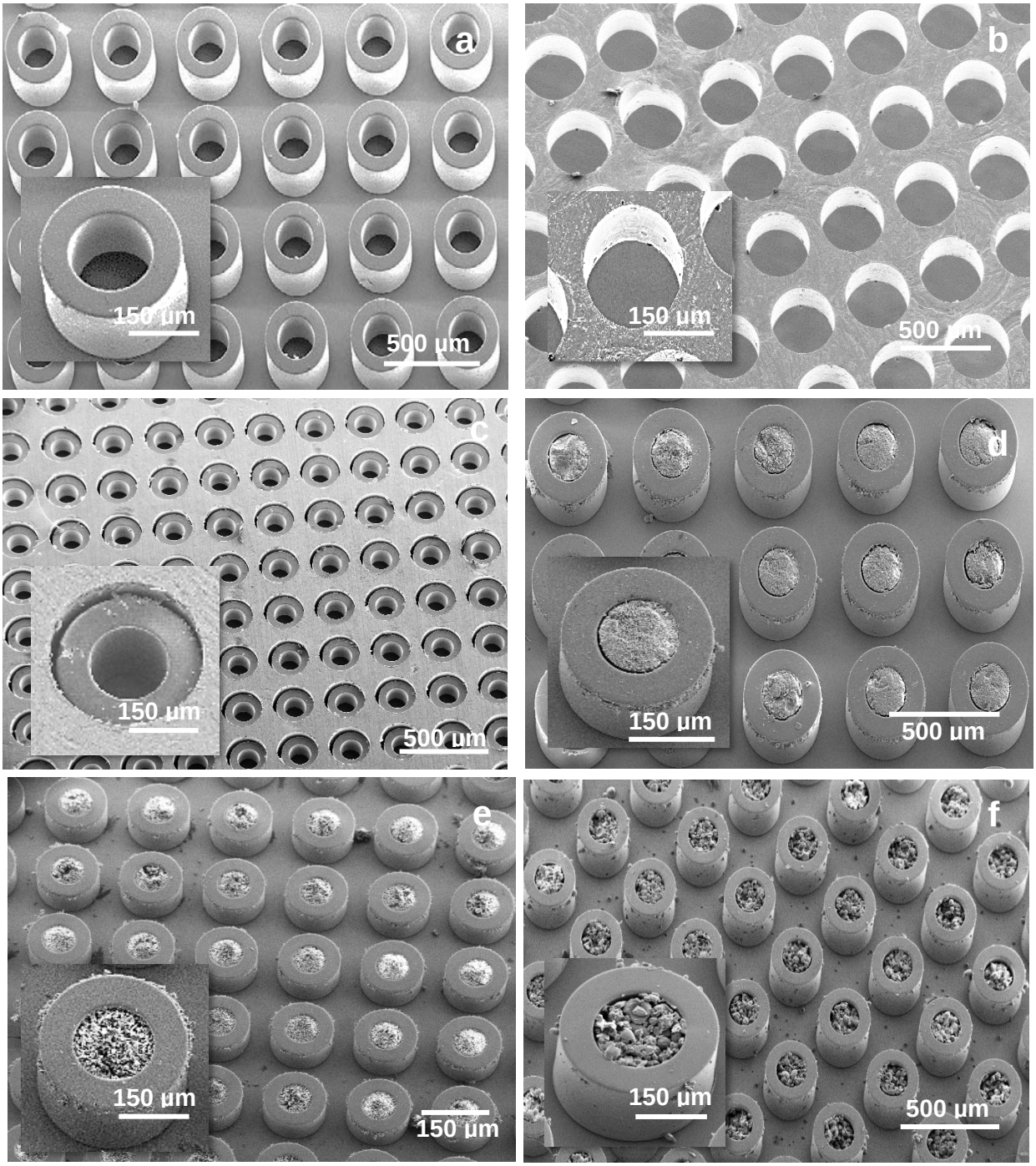

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Figure 3 SEM micrograph showing arrays and zoom images in the inserts of: a) SU-8 containers of 300 µm outer diameter and 300 µm height; b) the fabricated aluminum shadow mask with 380 µm circular holes; c) the microcontainers aligned with the fabricated shadow mask; microcontainers loaded with d) lipid-based microparticles, e) furosemide and f) PVP (K10) inside the container cavities using the powder embossing method

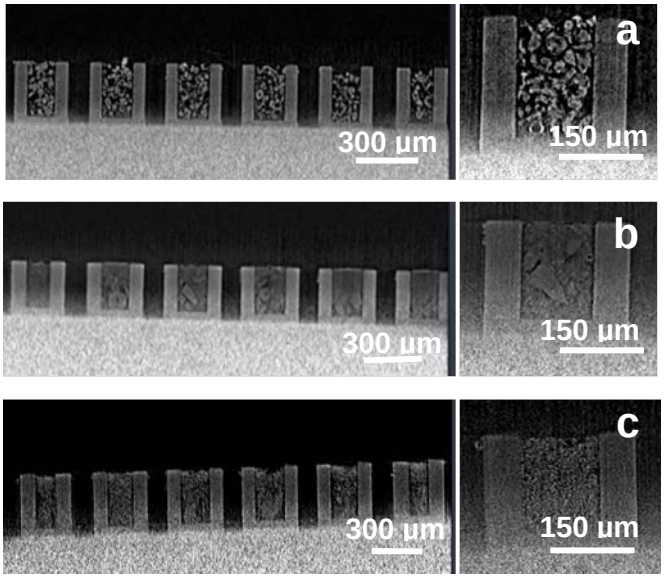

Figure 4 Cross-sections acquired by XµCT through the microcontainers filled with a) PVP (K10), b) lipid-based microparticles in the form of cubosomes, c) furosemide,

3.3 Release of furosemide from microcontainers

The release of furosemide from the containers was investigated in a medium with similar pH value as intestinal fluid (pH 6.5). Furosemide showed a two phased release (figure 5); the first phase was very rapid for 30 minutes where 40 % of the drug was released. This was expected as any possible loose powder on the top of the containers would be detected in the media almost simultaneously. Then, a slower diffusion rate occurred as the pressed powder from inside the containers was released. This rate continued until almost 100 % of the drug (1.5±0.2 mg/chip) was released from the containers. The fact that the signal was absorbed at UV wavelengths ranging from 310-350 nm indicates that the loading process did not affect furosemide at a chemical level as it would have absorbed at another wavelength otherwise. Also, the release curve is similar to other release studies of crystalline furosemide (data not shown). SEM micrographs of the containers after the release experiments confirm that the containers were emptied (Figure 6a). A few residues of powder were observed at the bottom of the containers reservoirs confirming the fact that the release profile did not reach exactly 100% of the weighed amount of drug. Similar release experiments were conducted for the containers loaded with lipid-based microparticles and PVP (Figure 6b-c). The micrographs after the microparticle release studies showed that significantly more powder residue was left after the release experiments which might be due to the dense filling and the sticky nature of the cubosomes. The empty PVP containers (Figure 6c) demonstrate that close to 100 % of the powder was released from the containers. Also, investigation of cubosomes in cryo-TEM before and after loading demonstrated that the microparticles were not affected by the pressure (data not shown).

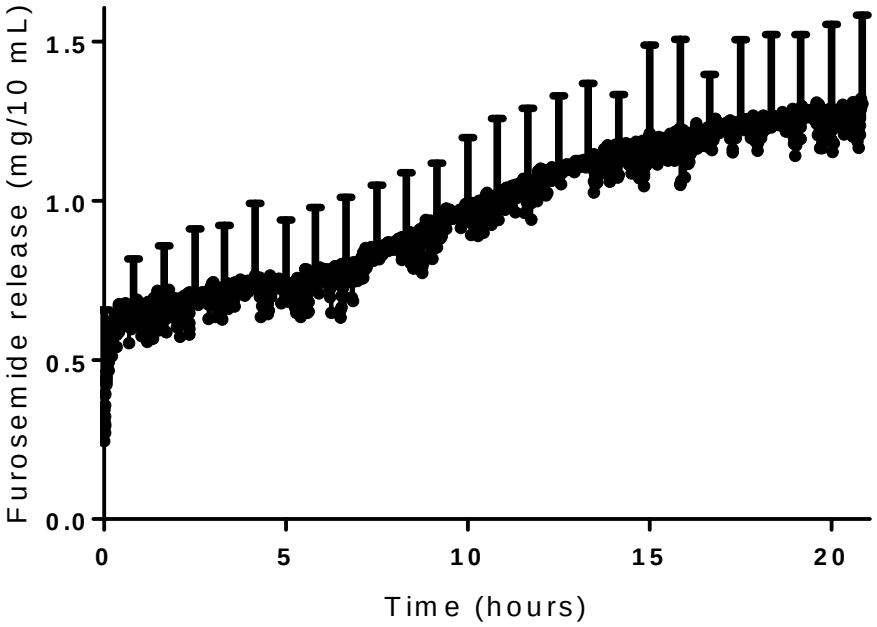

Figure 5 In vitro release profiles obtained from SU-8 microcontainers filled with furosemide in phosphate buffer (intestinal medium) at pH 6.5. Data is presented as average of 5 release studies +SD.

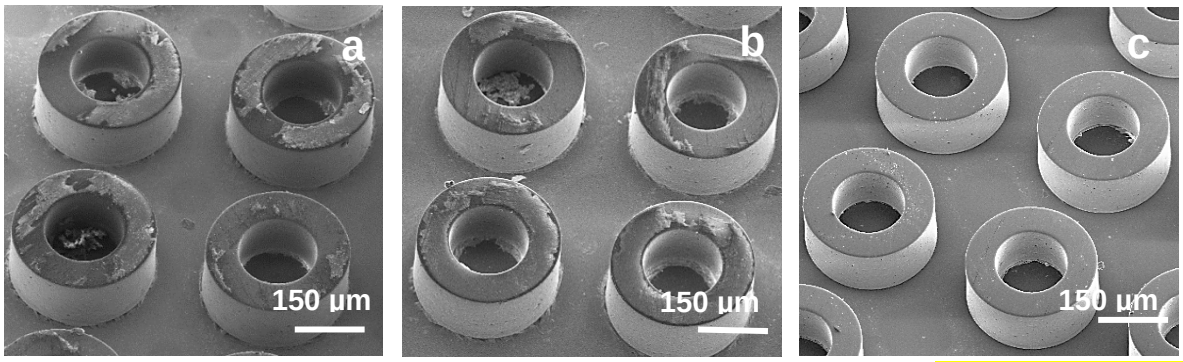

Figure 6 SEM micrographs of microcontainers after release in PBS pH 6.5 for 20 hours of a), furosemide b) lipid-based microparticles in the form of cubosomes, and c) polyvinylpyrollidone.

4 Conclusion

Here, we introduced powder embossing as a new method for loading of powder into microcontainers for oral drug delivery. For this purpose, the successful fabrication of an aluminum shadow mask with micro-milling process had to be demonstrated. Alignment and clamping of the shadow mask around the containers prevented deposition of drug between the microcontainers and restricted drug loading to the microcontainer cavities. Application of pressure allowed loading the container reservoirs with various powder formulations such as furosemide, polyvinylpyrrolidone and lipid-based microparticles in the form of cubosomes demonstrating the versatility of the method. An excellent loading efficiency, homogeneity, and reproducibility was confirmed by weighing, SEM imaging and X-ray microtomography measurements. Furthermore, waste of drug powder was minimized. The yield for the powder embossing was 100% for simultaneous loading of 625 microcontainers. The throughput of the method can potentially be increased by fabrication of larger arrays of microcontainers and corresponding shadow masks.

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5 References

[1] J. Guan, H. He, L.J. Lee, D.J. Hansford, Fabrication of particulate reservoir-containing, capsulelike, and self-folding polymer microstructures for drug delivery, Small. 3 (2007) 412–418. doi:10.1002/smll.200600240.

[2] S. Sant, S.L. Tao, O.Z. Fisher, Q. Xu, N.A. Peppas, A. Khademhosseini, Microfabrication technologies for oral drug delivery, Adv. Drug Deliv. Rev. 64 (2012) 496–507. doi:10.1016/j.addr.2011.11.013.

[3] H.D. Chirra, T.A. Desai, Multi-reservoir bioadhesive microdevices for independent rate-controlled delivery of multiple drugs, Small. 8 (2012) 3839–3846. doi:10.1002/smll.201201367.

[4] H.D. Chirra, L. Shao, N. Ciaccio, C.B. Fox, J.M. Wade, A. Ma, T.A. Desai, Planar Microdevices for Enhanced In Vivo Retention and Oral Bioavailability of Poorly Permeable Drugs, Adv. Healthc. Mater. 3 (2014) 1648–1654. doi:10.1002/adhm.201300676.

[5] C.B. Fox, H.D. Chirra, T.A. Desai, Planar bioadhesive microdevices: a new technology for oral drug delivery., Curr. Pharm. Biotechnol. 15 (2014) 673–83. doi:10.1016/j.micinf.2011.07.011.Innate.

[6] L.H. Nielsen, A. Melero, S.S. Keller, J. Jacobsen, T. Garrigues, T. Rades, A. Müllertz, A. Boisen, Polymeric microcontainers improve oral bioavailability of furosemide, Int. J. Pharm. 504 (2016) 98–109. doi:10.1016/j.ijpharm.2016.03.050.

[7] K.M. Ainslie, R.D. Lowe, T.T. Beaudette, L. Petty, E.M. Bachelder, T.A. Desai, Microfabricated devices for enhanced bioadhesive drug delivery: Attachment to and small-molecule release through a cell monolayer under flow, Small. 5 (2009) 2857–2863. doi:10.1002/smll.200901254.

[8] K.M. Ainslie, C.M. Kraning, T. a Desai, Microfabrication of an asymmetric, multi-layered microdevice for controlled release of orally delivered therapeutics., Lab Chip. 8 (2008) 1042–1047. doi:10.1039/b800604k.

[9] R.S. Petersen, S.S. Keller, A. Boisen, Loading of drug-polymer matrices in microreservoirs for oral drug delivery, (2016) Accepted.

[10] P. Marizza, S.S. Keller, A. Müllertz, A. Boisen, Polymer-filled microcontainers for oral delivery loaded using supercritical impregnation, J. Control. Release. 173 (2014) 1–9. doi:10.1016/j.jconrel.2013.09.022.

[11] L.H. Nielsen, S.S. Keller, K.C. Gordon, A. Boisen, T. Rades, A. Müllertz, Spatial confinement can lead to increased stability of amorphous indomethacin, Eur. J. Pharm. Biopharm. 81 (2012) 418–425. doi:10.1016/j.ejpb.2012.03.017.

[12] M.M. Deshmukh, D.C. Ralph, M. Thomas, J. Silcox, Nanofabrication using a stencil mask, Appl. Phys. Lett. 75 (1999) 1631. doi:10.1063/1.124777.

[13] A. Greve, S. Dohn, S. Keller, A.L. Vig, A. Kristensen, C.H. Nielsen, N.B. Larsen, A. Boisen, Wafer scale coating of polymer cantilever fabricated by nanoimprint lithography, in: Proc. IEEE Int. Conf. Micro Electro Mech. Syst., 2010: pp. 612–614. doi:10.1109/MEMSYS.2010.5442334.

[14] S.S. Keller, F.G. Bosco, A. Boisen, Ferromagnetic shadow mask for spray coating of polymer patterns, Microelectron. Eng. 110 (2013) 427–431. doi:10.1016/j.mee.2013.03.029.

[15] L.H. Nielsen, T. Rades, B. Boyd, A. Boisen, Microcontainers as an oral vaccine delivery system for spray dried cubosomes, (2016) Submitted.

[16] L.A. Feldkamp, Practical cone-beam algorithm Sfrdr I _ f, Opt. Soc. Am. A. 1 (1984) 612–619.