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APPLICATION NOTE

FABRICATION PROCESSES OF DUAL COMPARTMENT MICROCONTAINERS FOR SEQUENTIAL DRUG RELEASE

by

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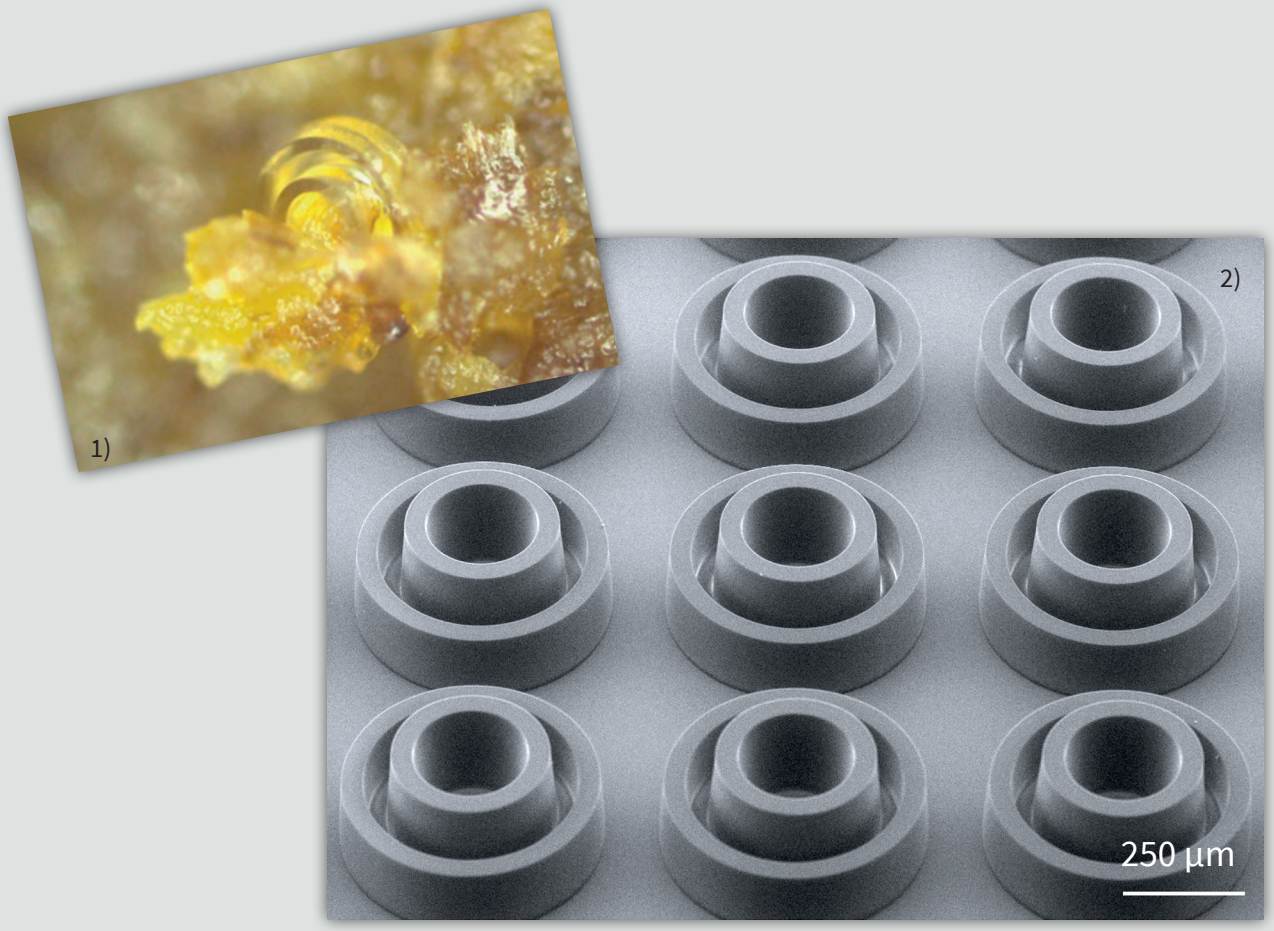

Combination drug therapy is commonly used to treat cancer, diabetes, cardiovascular conditions, and infections. However, these therapies face challenges associated with patient compliance and toxicology. Over the past decades, microdevices have emerged as a promising candidate for oral medication allowing for targeted drug delivery with a tuneable drug release.

Dual compartment microcontainers (DCMCs) were produced in the biocompatible negative photoresist SU-8 using a triple exposure scheme for defining the bottom, the outer compartment, and the inner compartment of the devices. The UV lithography was conducted on an MLA 100 maskless aligner from Heidelberg Instruments. After the resist development, the two compartments were loaded with different model drugs and selectively coated with enteric materials to produce thousands of discrete and monodisperse DCMCs capable of realizing sequential delivery in the gut during an in vivo experiment with rats. In addition to the produced DCMCs, a nickel shadow mask was made to facilitate manual drug loading into the inner compartment of the DCMCs. Said shadow mask was produced by electroplating from a scaffold substrate containing tapered SU-8 pillars made by UV lithography using the MLA 100 maskless aligner.

The governing hypothesis behind utilizing microscale drug delivery devices (DDD) is that they can enhance absorption of pharmaceutical compounds by increasing the retention time while simultaneously realizing a scenario where the active pharmaceutical ingredient (API) is delivered near the epithelium. Commonly, such microscopic devices are relatively simple and only produced to enable the release of a single API. The produced DCMCs allows for realizing co-delivery of two APIs in a sequential manner by separating the drugs physically in two compartments and by deposition of enteric coatings which dissolves at different rates upon entry into the small intestine. By utilizing the DCMCs, sequential delivery of two model drugs, furosemide, and propranolol, was demonstrated in vitro and in vivo. These proof-of-concept experiments could pave the way for combination drug therapy which is frequently used to treat cancer, diabetes, cardiovascular conditions, and infections.

DETAILED PROCESSES

Production of the DCMCs: The microscale containers were produced on a 525 µm thick Si carrier substrate which was coated with 5 nm Ti and 20 nm Au. The metal stack serves as an efficient release layer that allows for harvesting the produced DCMCs after API (active pharmaceutical ingredient) loading and enteric coating. The DCMCs are produced by sequentially exposing three SU-8 layers with different thicknesses as schematically outlined in Figure 1 (A). Each lithography step is composed of spin coating SU-8, solvent bakeout, UV exposure at 365 nm using the MLA 100 maskless aligner, and finally a prolonged low temperature (50°C) post exposure bake. Initially the 35 µm thick bottom layer is exposed using a dose of 250 mJ/cm^2. Hereafter, the sidewall of the outer cylindrical compartment is exposed in a 130 µm thick SU-8 layer using a dose of 350 mJ/cm^2. Finally, the sidewall of the inner compartment is exposed in the full depth of the three-layered SU-8 stack which has a thickness of approximately 250 µm. This exposure was carried out using a dose of 500 mJ/cm^2. The individual exposures were aligned by exposing suitable alignment marks in both the bottom and the outer sidewall layers. After the final lithography step, the DCMCs were developed by immersion in mr-Dev 600 in two separate baths. The immersion time in each bath was kept at 20 minutes. The DCMCs were designed as to have the same available loading volume in both the inner and the outer compartments.

The overall topography of the microscale devices was characterized using a combination of conventional bright-field optical microscopy and vertical scanning interferometry. Topography data can be found in Figure 1 (C) and additionally a scanning electron microscopy image has been included in Figure 1 (D). Prior to drug loading and coating, the Si substrate was diced out into 9 chips each containing 324 DCMCs arranged in a 18x18 array. Prior to in vitro and in vivo experiments, the inner compartment was loaded with API using a prefabricated nickel shadow mask. Hereafter, the first layer of enteric coating (Eudragit S100) was deposited using ultrasonic spray coating. A flexible PDMS masking approach was employed to ensure efficient API loading of the outer compartment and hereafter the second enteric coating layer (Eudragit L100) was deposited by ultrasonic spray coating. The enteric coatings ensure protection of the loaded APIs at gastric pH (i.e. pH < 2) while allowing for triggered release when the devices enter the small intestine where the pH is elevated (i.e. pH > 6). Prior to animal experiments, the DCMCs were harvested and loaded into size 9 gelatin capsules.

Figure on front page: 1) Optical microscopy image of empty DCMCs embedded into the mucus and intestinal content in the ileum. 2) Scanning electron microscopy image showing the final DCMCs. The outer diameter of the outer compartment is approximately 520 µm.

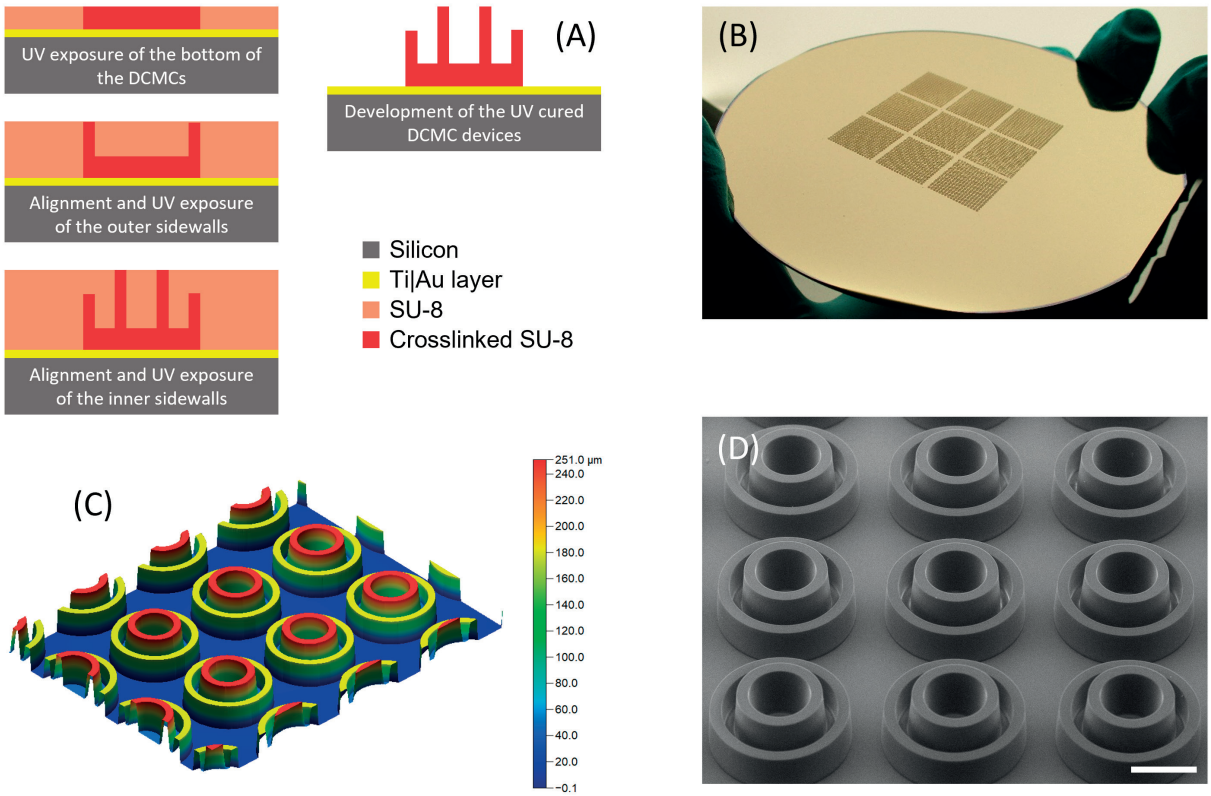

Figure 1: Schematic showing the process flow with pictures and images showing the final DCMC devices. (A) The process flow consists of three UV lithography steps in which the DCMC devices are gradually defined by MLA exposures. The final device features concentric cylindrical compartments and the DCMCs are made on a Ti|Au layer which allows for detaching the containers after drug loading and enteric coating. (B) Picture showing the 4" Si substrate with 9 discrete fields each containing 324 DCMCs arranged in a 18x18 array. (C) 3D contour plot based on vertical scanning interferometry measurements on the devices. The DCMCs feature a 35 µm thick bottom and the inner heights of the two compartments are 130 µm (outer compartment) and 215 µm (inner compartment) respectively. (D) Scanning electron microscopy image showing the final DCMCs. From the image it is evident that the sidewalls are slightly tapered. The outer diameter of the outer compartment is approximately 520 µm. The scalebar corresponds to 250 µm.

PRODUCTION OF THE NICKEL SHADOW MASK

The nickel shadow mask, used for API loading into the inner compartment, was made by electroplating on a substrate containing a predefined scaffold of slightly tapered (taper angle of approximately 85°) cylindrical SU-8 pillars having a height of 250 µm and a diameter of 330 µm at the top surface. Additionally, markers used for alignment and laser cutting were also defined. A schematic of the process flow has been included in Figure 2 (A). Starting out with a 525 µm thick silicon substrate, a 1.5 µm thick resist mask for lift-off was made by tone inversion in the positive resist AZ®5214 E. Initially, the MLA 100 maskless aligner was used for exposing the inverse pattern at a modest dose of 40 mJ/cm^2. Hereafter, the substrate was subject to an inversion bake at 110°C for 120 s, followed by flood exposure in a conventional mask aligner using a dose of 240 mJ/cm^2 and finally single puddle development in AZ®726 MIF for 120 s. Next, a suitable seed layer consisting of 10 nm Ti and 100 nm Au was deposited using E-beam evaporation and lift-off was conducted in a bath with Remover 1165. Following the production of the prepatterned seed layer, 250 µm thick SU-8 was spin coated and subject to solvent bakeout, UV lithography using the MLA 100 maskless aligner and a dose of 550 mJ/cm^2, post exposure bake and finally development in two dedicated baths containing mr-Dev 600. After development, the substrate was subject to thorough rinsing in isopropanol. The nickel shadow mask was then

This application note describes the fabrication processes of the micro-containers used in the paper "Sequential Drug Release Achieved with Dual-Compartment Microcontainers: Toward Combination Therapy" published by Advanced Therapeutics, accessible here: https://onlinelibrary.wiley.com/doi/10.1002/adtp.202270024

electroplated to a final thickness of 200 µm by carefully setting the setpoint charge associated with termination of the electroplating process. The carrier silicon substrate was then etched away in a 50 wt% KOH solution heated to 85°C. After this step, the nickel shadow mask needed a final clean to remove the SU-8 pillars that were still lodged inside the holes of the shadow mask. This final cleaning step was conducted in a piranha solution composed of H_2SO_4 mixed with H_2O_2 in a 4:1 (H_2SO_4:H_2O_2) ratio. The nickel shim was then subject to laser micromachining for cutting out the individual shadow masks. A picture of one of the shadow masks has been included in Figure 2 (B) and scanning electron images showing the tight fit of the mask to the DCMCs and the drug loading efficiency has been included in Figure 2 (D). The laser cut shadow masks were then finally cleaned using sonication in DI water with Triton X-100 followed by sonication for 20 min in isopropanol.

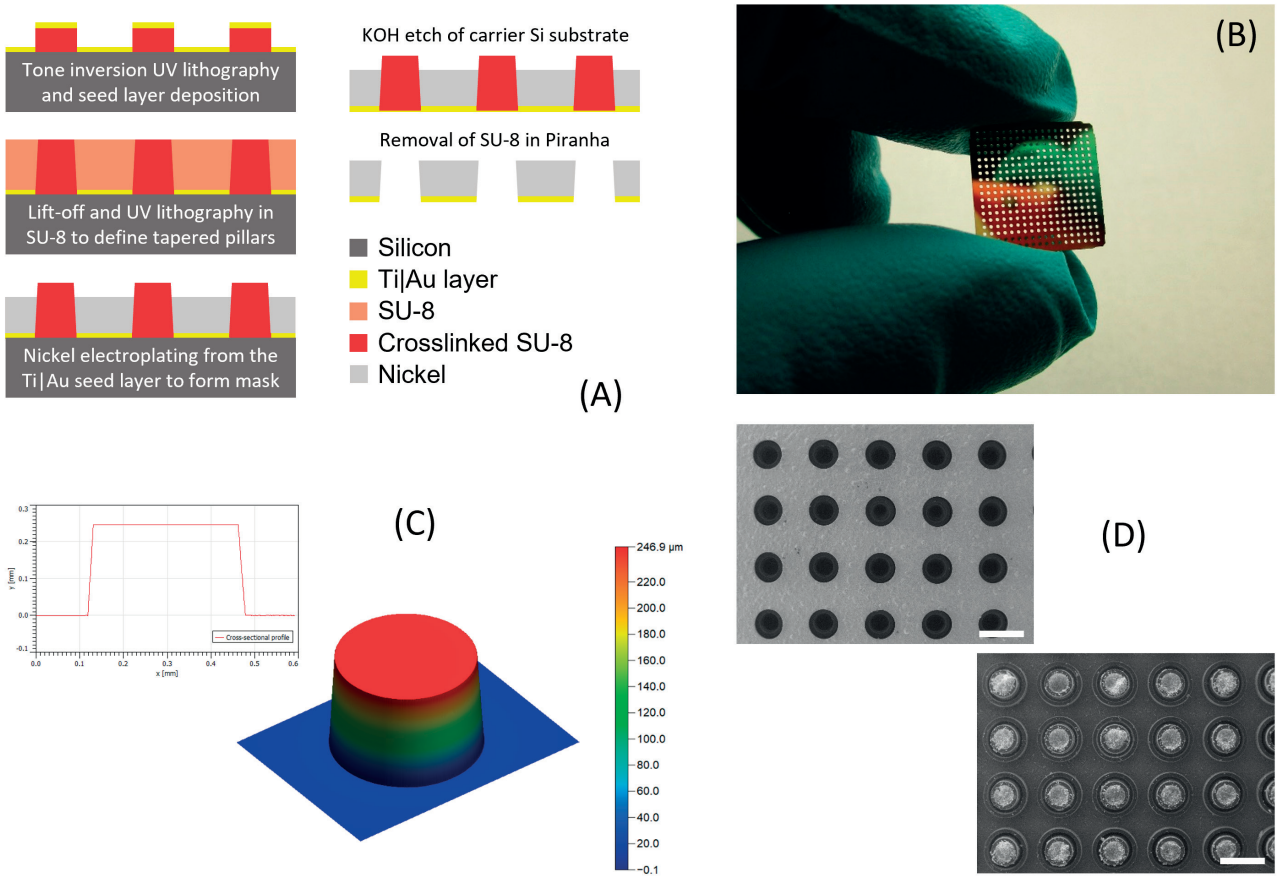

Figure 2: Schematic showing the process flow for making the electroplated nickel shadow mask with pictures and images acquired during characterization and actual use of the mask for drug loading. (A) The process flow consists of UV lithography with tone inversion in combination with metal deposition and lift-off for making the seed layer. Hereafter, UV lithography in thick SU-8 is employed to make tapered pillars that dictate the size and geometry of the circular holes in the shadow mask. By nickel electroplating, the shadow mask is formed, and both the Si substrate and the SU-8 pillars are selectively removed prior to laser micromachining and extensive cleaning. (B) Picture of one of the final nickel shadow masks used for loading API into the inner compartment of the DCMCs. (C) 3D contour plot and cross-sectional profile of one of the SU-8 pillars. Notice the 85° taper angle of the sidewalls which ensures a tight fit to the DCMCs during drug loading. (D) Scanning electron microscopy images of the shadow mask mounted onto a chip with DCMCs (top image) and the micro-devices after successful loading of drug inside the inner compartment. The scalebars in both images correspond to 500 µm.

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