

## Spatially Controlled Chemistry Using Remotely Guided Nanoliter Scale Containers

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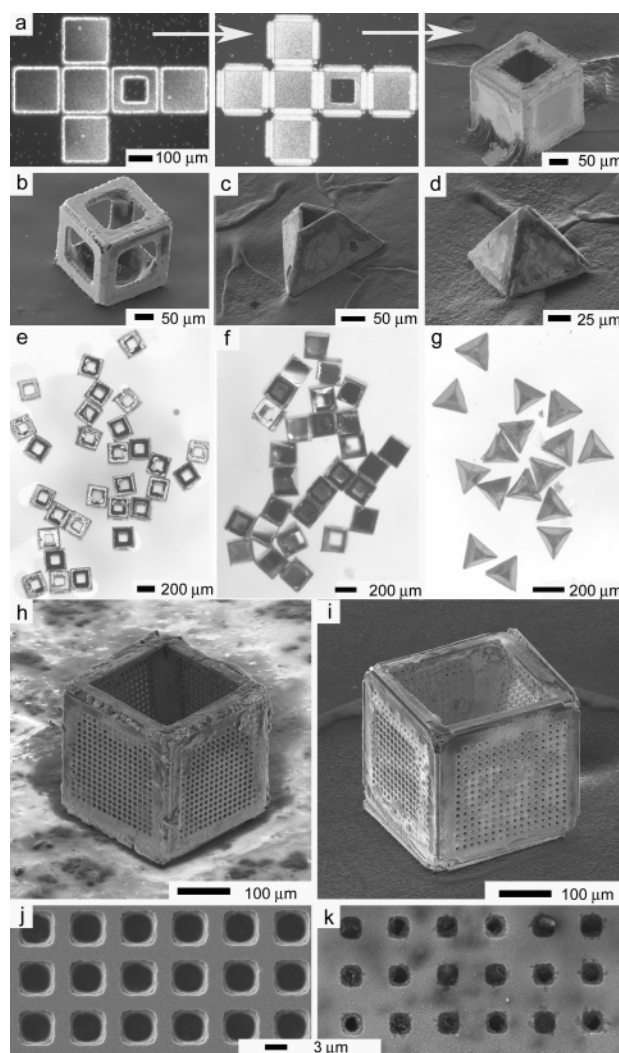
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In this communication we describe a new pico-nanoliter scale chemical encapsulation and delivery system in the form of 3D metallic containers with controlled surface porosity. The advantageous attributes of the containers are a parallel fabrication process with versatility in size and shape, precise and monodisperse surface porosity, and the ability for remote guidance using magnetic fields. We demonstrate the isotropic and anisotropic release of reagents from the containers, remotely guided chemical release, and spatially controlled chemical reactions in microwells. The arbitrary chemical patterns that can be generated are not limited by flow profiles in conventional microfluidics that must often be formed downstream from a channel inlet. These containers suggest a new strategy for precisely engineering spatially localized chemical reactions with subnanoliter scale control.

In the past decade, microtechnology has greatly impacted the chemical and biological sciences with the development of microfluidic systems that are capable of manipulating small volumes of reagents. Microfluidic devices make it possible to execute and observe chemical reactions with spatial and temporal control. The applications of microfluidics include the measurement of enzyme reaction kinetics,<sup>2</sup> capillary electrophoresis,<sup>3</sup> flow cytometry,<sup>4</sup> PCR amplification,<sup>5</sup> immunoassays,<sup>6</sup> mass spectrometry,<sup>7</sup> DNA analysis,<sup>8</sup> cell patterning,<sup>9</sup> and cell separation.<sup>10</sup>

Along with conventional channel based microfluidic devices, several nanoliter scale chemical encapsulants have been developed, including those based on polymers, gels, and liquid drops.<sup>11</sup> In contrast to the above organic systems, micromachined silicon-based devices can have extreme precision, high reproducibility, excellent mechanical strength, good chemical stability, as well as the ability to incorporate sensing, signal conditioning, and actuating functions in close proximity or on the same substrate. However, 3D micromachined nanoliter scale reservoir systems with controlled porosity do not exist at the present time owing to the inherent two-dimensionality of the photolithographic process that is used in conventional silicon-based micromachining.

Here, we describe the development of 3D containers with precisely engineered surface porosity and their utility in chemical encapsulation, guided delivery, and spatially controlled chemistry. Briefly, the process involved the photolithographic fabrication of a 2D metallic template with solder hinges (Figure 1a). The 2D template self-assembled into the 3D hollow polyhedron when it was heated above the melting point of the solder hinges, wherein the surface tension of the molten solder provided the force to drive self-assembly.<sup>12</sup> (see Supporting Information (SI) for details). We fabricated containers with different shapes and volumes ranging from 230 picoliters to 8 nanoliters (Figure 1a–d). The fabrication process was also highly parallel; containers of different shapes and sizes could be fabricated in a single process run (i.e., from a single wafer, Figure 1e–g). When the process was optimized, yields

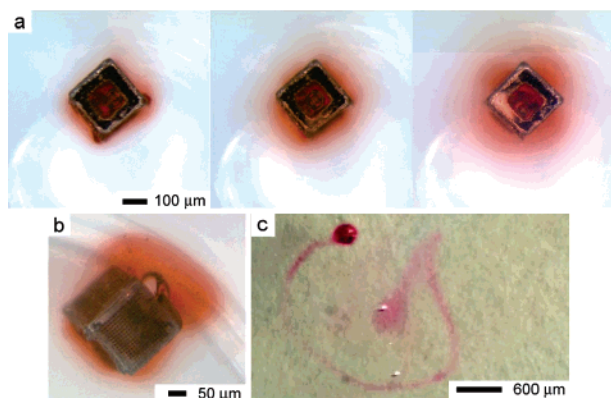


**Figure 1.** (a) Optical and SEM images showing the different steps (the photolithographically fabricated 2D template, registry of solder hinges, and the folded 3D structure) in the fabrication of a cubic container with one open face. SEM images of a (b) cubic container with all open faces, (c) pyramidal frustum, (d) square pyramid with an open face on the bottom; (e–g) optical image of multiple containers of different shapes demonstrating the parallel fabrication strategy; (h–k) SEM images of cubic containers with monodisperse pore sizes of (h, j) 5 microns and (i, k) 3 microns.

ranged from 60 to 90% (yields varied for different shaped containers depending on the number of folding faces and the symmetry) for a 3" wafer. The major yield limiters in the fabrication process were the photolithographic fidelity in the registry of hinges with respect to the faces and the volume of solder in the hinges.<sup>13,14</sup> Since photolithographic microfabrication is highly precise, it was also possible to pattern one or more faces of the containers with

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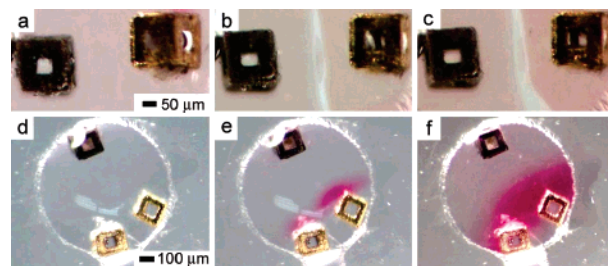
**Figure 2.** Optical images of chemical release from containers: (a) spatially isotropic release of a dye from a container with identical porosity on all faces; (b) anisotropic release of a dye from a container with anisotropic porosity (five faces with an array of 5 micron pores; the sixth face has a 160 micron sized pore); (c) an example of a remotely guided spatially controlled chemical reaction. The letter G (for the Gracias lab, see video in SI) was formed by the direct writing of phenolphthalein in an alkaline water–glycerol medium.

monodisperse pores (Figure 1h–k). The size of the pores formed was limited by the photomasks used (which in our case had a resolution of 3 microns). By controlling the porosity, it was possible to engineer the reagent release profiles as shown in Figure 2.

We loaded containers using stereotactic microinjection. We loaded containers with a solution of a gel (or polymer) and the chemical to be released. When the solvent evaporated, the gel remained within the containers. The chemicals were released by immersing the loaded containers in a solution that softened or dissolved the gel (or polymer). Since gels (and polymers) are available with a wide range of solubility and softening temperatures, it was possible to manipulate the chemical release rates using different solvents and temperatures. The images shown in the paper were obtained using containers loaded with a block copolymer hydrogel (Pluronic). Release experiments were done in a water–alcohol based medium (details in the SI). By varying the relative porosity on different faces of the container it was possible to get both isotropic (Figure 2a) as well as anisotropic (Figure 2b) chemical release profiles.

Since the fabrication process was compatible with a variety of materials, it was possible to fabricate nickel based containers that could be remotely guided using magnetic fields. We demonstrated a spatially controlled chemical reaction by directly releasing (writing) a pH indicator phenolphthalein in a microwell filled with an alkaline solution (Figure 2c), to write the letter G (any arbitrary trajectory is possible, video included in SI). Direct writing was possible by manipulating the phenolphthalein–pluronic loaded container using a magnetic stylus that was moved under the microwell. It should be noted, that while our guided manipulation was done using a permanent magnet, it is possible to use other well-developed microcoil based magnetic manipulation circuits<sup>15</sup> to reproducibly control the movement of the containers and hence the spatial release of the chemicals with arbitrary patterns.

We also visualized spatially localized chemical reactions between multiple nanoliter scale containers (Figure 3a–c). When two containers loaded with copper sulfate and potassium hydroxide, respectively, were brought close to each other in an aqueous medium, a chemical reaction (to form copper hydroxide) occurred only along the central line between the two diffusing reactants (which appear to diffuse at roughly the same rate). When the chemicals reagents diffused out of the containers at different rates, the reaction occurred nearer the containers with the slower diffusing



**Figure 3.** Spatially controlled chemical reactions between multiple containers: (a–c) reaction of copper sulfate and potassium hydroxide in an aqueous medium resulting in the formation of copper hydroxide along the central line between the containers; (d–f) the reaction of phenolphthalein (diffusing out of the two bottom containers) and potassium hydroxide (diffusing out of the top container) in an aqueous medium.

chemical (Figure 3d–f). These experiments further demonstrate that the spatial control over chemical reactions can be extended to more complex reaction fronts involving multiple containers.

In conclusion, as opposed to all organic encapsulants, the containers allow unprecedented spatial control over the release of chemical reagents by virtue of their versatility in shapes and sizes, anisotropic faces, monodisperse porosity, and their ability to be guided in microfluidic channels using magnetic fields. Additionally, the metallic containers interact with remote electromagnetic fields that allow them to be easily detected and tracked (using magnetic resonance imaging, MRI) as demonstrated in a previous paper.<sup>12c</sup> Thus, the containers provide an attractive platform for engineering remotely guided, spatially controlled, chemical reactions in microfluidic systems.

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**Supporting Information Available:** Details of the methods used in the fabrication, loading, release, chemical reactions, and a video showing the direct writing of the letter G. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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