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Remote Radio-Frequency Controlled Nanoliter Chemistry and Chemical Delivery on Substrates**

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The field of microfluidics has revolutionized chemical analysis and has led to the proposal of new paradigms for cell engineering and drug delivery.[1] Nanoliter-scale chemical systems including lab-on-a-chip devices, microfabricated capsules, and polymeric spheres make it possible to execute chemical reactions with spatial and temporal control. The advantages of these devices include minimal size, low fabrication costs, efficient use of reagents, good reproducibility, precise volumetric control, and high parallelism. For the first time, we have combined the favorable attributes of microfabricated nanoliter-scale fluidic devices with wireless technology by fabricating remote-controlled cubic containers. As opposed to electrically wired systems, wireless technologies facilitate the fabrication of convenient, mobile, autonomous, and non-invasive devices. Previous demonstrations of wireless radio-frequency (RF) technology in nano- and sub-nanoliter-scale chemical engineering involve the remote heating of metal nanoparticles or polymer magnetic microspheres.[2] In contrast to these systems, microfabricated containers have a reproducible volume for the encapsulation of chemicals, excellent mechanical and chemical stability, and homogeneity in physical characteristics. We are able to use a single fabrication methodology, which is compatible with the techniques used to pattern microelectronic devices, to construct containers of different materials, size, shape, and porosity.[3] By exploiting well-established lithographic methods in microelectronics, our fabrication process provides a route to incorporate transistors, sensors, and other information-processing devices onto the containers. Moreover, since our containers completely encapsulate their contents, they can be easily moved on substrates without sticking or leaving a residue (which can occur in polymer magnetic microspheres), and hence allow for true spatial control of chemistry on substrates. Polymeric magnetic microspheres can also cause the release of the magnetic particulate (along with the chemicals) on collapse, which can be deleterious to cells.

We fabricated containers out of metal, which allowed them to be remotely coupled to electromagnetic sources; we utilized this feature to enable wireless control over both the spatial guidance (using magnetic containers) as well as the delivery of nanoliter volumes of chemical reagents. The containers can be guided in spatial patterns that are not limited by flow profiles in conventional microfluidics, that is, downstream from a channel inlet. We demonstrate that the remote-controlled nanoliter containers enhance the capabilities of present-day microfluidics by enabling spatially controlled chemical reactions, microfabrication within capillaries, and on-demand localized delivery of chemicals to cultured cells.

We used a combination of conventional microfabrication and self-assembly[3,4] to fabricate gold-coated, nickel nanoliter containers (Figure 1a). To facilitate chemical delivery, the containers were filled with a gel that was soaked in the chemical reagent to be released (Figure 1b). We utilized two gels: pluronic[5] for general dry-release experiments and poly(*N*-isopropylacrylamide) (PNIPAm)[6] for chemical delivery in aqueous solutions and to living cells. Pluronic is a water-soluble block copolymer hydrogel that softens at 52 °C and is compatible with a wide range of chemicals.[5] Hydrogels based on PNIPAm[6] are thermoresponsive materials that are widely used in drug delivery, because they undergo a structural transition near the temperature range of the human body.[7] This transition temperature, as well as the collapse kinetics of PNIPAm, can be altered by adding co-monomers and changing the degree of cross-linking.[8] Hence, PNIPAm is an ideal candidate for remote-controlled release to living cells and in liquid media.

Once loaded, a container was placed in the reaction vessel of choice and could be guided in any spatial trajectory using a magnetic stylus. After guidance to the desired location, a radio-frequency (RF) field, generated by a 2D microcoil, was directed towards the container. The power in the RF field coupled inductively to the metallic container, thereby producing eddy currents in the frame and heating it up by a Joule effect. It is possible to heat even nonmagnetic metallic

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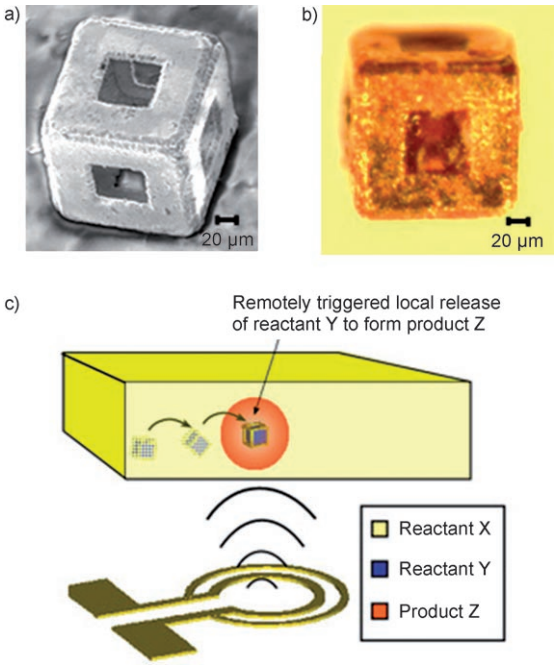

Figure 1. a) A scanning electron microscope image of an empty container. The containers were three-dimensional (3D) porous cubes with a length of approximately 200 μm and a volume of 8 nL. b) An optical microscope image of a container loaded with a dye-soaked pluronic gel. c) A schematic diagram of the experimental set-up used to facilitate wireless microscale chemical engineering (not drawn to scale). Containers were manipulated using a magnetic stylus (not shown) and the contents of specific containers were released by directing an RF source towards the container. In the schematic representation, chemical Y is released from a specific container; chemical Y then reacts with chemical X in the surrounding medium to form product Z.

containers by inductive coupling, and the heating mechanism is different from that used to heat polymeric magnetic microspheres. Since the containers were microfabricated, the electrical characteristics could be made reproducible, and the temperature could be precisely controlled by changing the incident power. This reproducibility should be contrasted with the power needed for release from polymeric magnetic microspheres, which can vary greatly because of polydispersivity in sizes and inhomogeneous distribution of magnetic particles within different microspheres.

By heating the container, the gel encapsulated within it softened (or collapsed) and released the chemical at the targeted spatial location (Figure 1c). The metallic containers are essential to obtain heating at the power and frequency settings used. No release was observed from the gel in control experiments (on exposure to the RF radiation, but in the absence of the container) because of negligible dielectric heating at the frequency and power setttings used (see the Supporting Information).

The remote-controlled containers make it possible to do chemistry with unprecedented spatial control in hard-to-reach regions. To highlight this feature, we repaired a break gap in one of two adjacent microwires embedded within a capillary; the capillary was accessible only by input and output ports (Figure 2). The gap within microwire 1 was repaired by remotely guiding containers to that site in air (Figure 2a,b) and remotely releasing first a chemical sensitizer and then an activator (using two separate containers) locally at the site of the gap (Figure 2c). The sensitizer and activator were tin and palladium catalysts, respectively, which facilitated the electroless deposition of copper. After sensitizing and activating the spatial region within the gap of microwire 1 only (Figure 2d), the entire capillary was flushed with a commercial solution of copper sulfate (Figure 2e). Although both microwires and the walls of the capillary were exposed to the copper sulfate solution, metallic copper deposited only at the chemically sensitized and activated gap in microwire 1 (Figure 2f). Electrical resistivity measurements confirmed electrical continuity of microwire 1 across the gap. This result demonstrates the utility of the containers for localized chemical delivery and chemistry within capillaries and other small spaces. In comparison to already

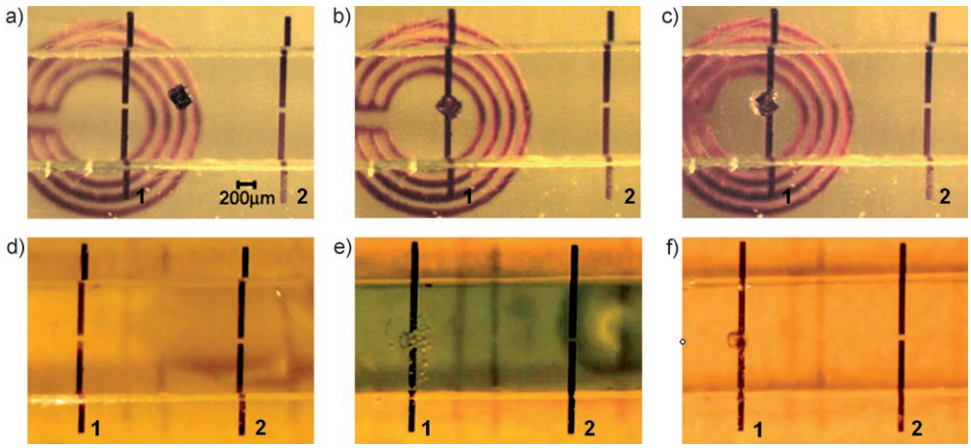

Figure 2. Optical images showing the remote controlled, spatially localized microfabrication within a capillary. Two microwires (1 and 2) were embedded within a microfabricated capillary (ca. 1 mm in diameter and 1.5 cm in length) and the capillary was aligned on top of a 2D microcoil. a, b) First, a container filled with pluronic and soaked with the chemical sensitizer was guided into the capillary to the site of the gap within wire 1 using a magnetic stylus. c) The chemical sensitizer was released by remotely heating the sensitizer-soaked pluronic gel that was encapsulated within the container. This heating was achieved with the 2D RF coil. After sensitizing the gap, the first container was removed, a second container was guided to the same gap in microwire 1, and the activator was released by heating the pluronic gel remotely. d) After activation, the second container was also removed. e) The capillary was then flushed with a commercial electroless copper-plating solution; chemical reduction (bubbles of the hydrogen gas, a by-product in the reaction, can be seen) of copper sulfate to metallic copper, occurred at the gap within microwire 1. f) Copper was deposited only in the gap between microwire 1, no copper was deposited in the gap in microwire 2.

existing methods of microfabrication in capillaries,[9] our method is not limited by the geometry of the capillary or laminar flow profiles.

A second demonstration highlights the utility of the nanoliter containers in the remote-controlled, localized delivery of sub-nanoliter volumes of chemicals to specific cells cultured on substrates. Containers were loaded with PNIPAm soaked in a live/dead (green/red) two-color fluorescence viability stain[10] to stain cells locally in a culture dish and to verify that no necrotic cell death occurred during chemical release as a consequence of the heating[11] or exposure to the RF radiation.

The L929 mouse fibroblast cells were cultured in 35-mm well-plates with glass inlays and grown to confluency. At the start of the remote-release experiment, the growth media was removed and the cells were rinsed with phosphate-buffered saline to dilute the serum esterase activity, thereby minimizing background fluorescence. To enable remote release of the stain, an RF coil was placed below the plate directly under a container, and the coil was powered up at 2–3 W for 1 minute to collapse the encapsulated PNIPAm and release the stain. Fluorescent images were obtained 30–60 minutes after release, to allow sufficient time for uptake of the stain. It is clear from the confocal fluorescence images (Figure 3) that the stain was released locally and within a radius of less than 500 μm from the center of the container. It can also be seen that the cells exposed to the stain had green fluorescence, thus indicating that they were alive, and no red fluorescing or dead cells were observed. The results indicate that neither the temperature used to collapse the encapsulated PNIPAm nor the RF radiation caused necrotic cell death. It should be noted that no leakage or spontaneous release (that is, no cell staining) was observed from the containers in experiments where no RF field was applied (see the Supporting Information for details). We have additionally performed biocompatibility studies that show no necrotic cell death occurs in the presence of the containers over 48 h.

In conclusion, the metallic, self-assembled nanoliter containers can be utilized for remote-controlled microfabrication and chemical delivery in hard to reach spaces. We believe that the containers will be useful in fabricating complex and reconfigurable microanalytical, microfluidic, and micro-electromechanical systems. The localized remote delivery of chemicals to cells establishes a methodology for remotely manipulating the chemical and biological microenvironment for applications in cell engineering, tissue engineering, and drug development. Finally, the containers provide an attractive platform for the integration of additional features of wireless devices (for example, frequency-selective remote control and remote communication) with the delivery of nanoliter volumes of chemicals.

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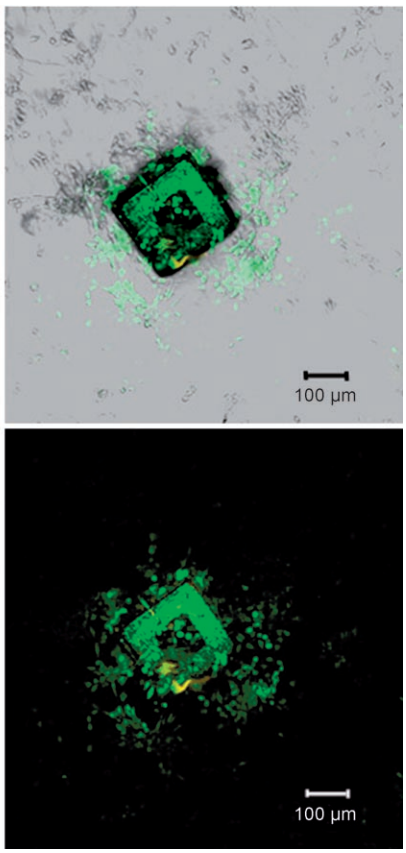

Figure 3. Cell-viability assessment by live/dead fluorescent imaging of calcein AM and ethidium homodimer-1, both released remotely from the containers. a, b) Confocal images of the local release of the live/dead stain to L929 mouse fibroblast cells. No red cells were observed, thus indicating no necrotic cell death during the release. a) Transmitted light differential interference contrast (DIC) images showing both the cells and the container. b) Fluorescent image showing only localized cell staining.

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