Development of nanoporous picoliter reaction vessels for the characterization of biochemical systems

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Incredibly complex biochemical reactions are carried out in living cells with tremendous effectiveness. This is due, in great part, to the confinement of the cells internal machinery within a reaction volume that allows for efficient molecular transport and mixing of the reaction volume via diffusion and the capacity of the cell to exchange chemical information with its local environment. In pursuit of the development of a synthetic platform for carrying out biochemical reactions that combines the virtues of small volume containment and selective chemical transport with the robustness and versatility of inorganic materials we have developed nanoporous reaction vessels using a combination of electron and photolithographic techniques with cryogenic silicon etching (Fig 1). These picoliter volume vessels facilitate the spatial confinement of large biomolecules in solution while allowing the exchange of small molecules with the surrounding microenvironment.

Electron beam lithography and cryogenic etching are used to define the container membrane allowing the deterministic control of the high aspect ratio pore width. Membranes with pores having widths as small as 35nm have been fabricated successfully. Performing hybrid lithography, using both electron beam and photolithography, makes it possible to define the fluidic channel and nanoporous container in a single step. Altering lithographic patterns, used to define the container membrane, or adjusting the duration of the cryogenic etching process afford control of the overall container volume.

Filling of the containers with reaction constituents has been performed using a classical cell microinjection system. Pulled glass pipettes with tip diameters of 5 μm are used to serially place reagents within the devices, which are then sealed with a silicone elastomer lid. The remainder of the reaction constituents are then delivered via the integrated microfluidic network. The confinement and labeling of DNA with ethidium bromide and the performance of a coupled horseradish peroxidase and glucose oxidase reaction have been demonstrated.

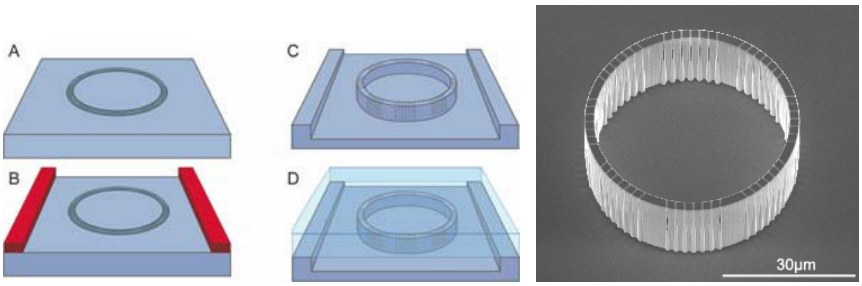

Fig 1 – *Building a Nanoporous Reaction Vessel:* (A) electron beam lithography followed by metal liftoff of a chrome mask is used to define the container membrane geometry. (B) Optical lithography is then used to define the microfluidic channel geometry. (C) Cryogenic etching transfers the lithographically defined patterns into the exposed silicon. (D) Following removal of the masking material the structure is coated with silicon dioxide and sealed with polydimethylsiloxane (PDMS).

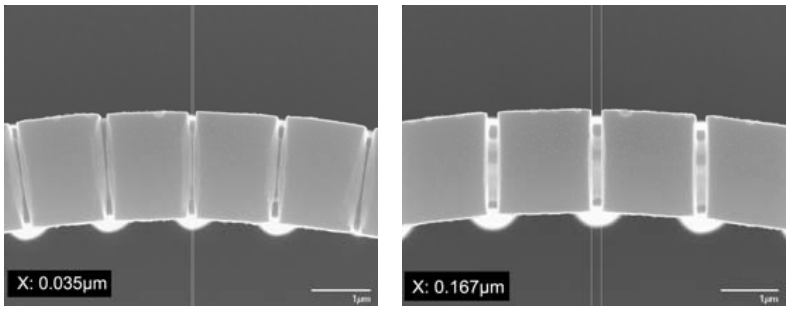

Fig 2 – *Nanopore configuration:* A close up view (18,000x) of two membranes structures demonstrates that the membrane geometry can be readily varied to control pore width, spacing, geometry and orientation. (X: pore width)

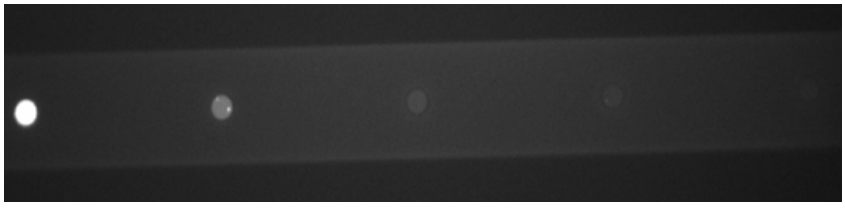

Fig 3 – *DNA confinement and labeling:* A ten fold serial dilution of a 5100 bp plasmid was added to a set of picoliter containers using a cell microinjection system fit with a five micron diameter pulled pipette. The device was sealed and washed with solution of ethidium bromide and imaged under flow using an epifluorescence microscope and digital CCD camera. The center to center spacing between the reaction vessels is 400 µm.