Fabrication of Shape-Specific, Enzymatically-Triggered Nanoparticles using Nano Imprint Lithography

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Statement of Purpose: Current concepts in the development of nanocarriers primarily involve the use of polymers or lipids to fabricate self-assembled or emulsion-based particles that are mostly spherical, polydisperse, and release drugs through diffusion or hydrolysis. Despite significant progress in such drug delivery systems, there remain critical limitations in synthesizing nanocarriers with highly controllable architecture (size, shape or aspect ratio) that can, at the same time, impart environmentally-triggered release mechanisms. These parameters are essential for controlling the *in-vivo* transport, biodistribution, and drug release mechanism of nanoparticles. Our objective is to use Step and Flash Imprint lithography (S-FIL) to fabricate stimuli-responsive, easily harvestable nanoparticles (as small as 50 nm) of precise sizes, shapes, and compositions. Applying S-FIL technology, our group has fabricated a variety of shape and size nanoparticles that are environmentally response sensitive [1]. The particle matrix incorporates enzymatically-degradable acrylated peptides and acrylated polyethylene glycol macromers and can provide triggered release of encapsulated drugs or contrast agents in response to specific physiological or pathophysiological conditions.

Methods: We have investigated the use of S-FIL based fabrication of nanocarriers using photo-crosslinkable macromers; PEG diacrylates (PEGDA) as previously reported [1]. A combination of PEGDA and acrylated, enzymatically degradable GFLGK-DA was used to produce stimuli-responsive particles, such that the cross-linked nanoparticle-matrix is degraded in the presence of tumor-specific enzymes. S-FIL was performed using the IMPRIO 100 S-FIL system (Molecular Imprints, Austin TX) For efficient, one-step particle harvesting from the imprinting surface, a layer of water soluble poly(vinyl alcohol) (PVA, Fluka, Mw ~30,000 Da) is applied prior to imprintation. A critical aspect of using such imprinting techniques to generate nanocarriers is our ability to efficiently and easily harvest intact particles from the silicon wafer. S-FIL fabricated imprints were plasma etched, incubated in filtered dH_2O, and gently pipetted to dissolve the PVA layer thus releasing the nanoparticles. We have further demonstrated synthesis of nanocarriers that can be degraded only in the presence of specific intracellular or tumor-specific enzymes [1]. The surfaces of these nanocarriers are functionalized with cell targeting ligands. Further studies evaluating intracellular drug delivery and enzyme-triggered drug release are currently underway.

Results: Figure 1 A-F shows S-FIL generated arrays of PEGDA (Mw 700) nanoparticles. The various panels underscore the power of this method in generating highly monodisperse nanoparticles of precise sizes and geometries. As shown, particles with square (a, c, d), triangular (b) as well as particles with high aspect ratio (e, f) profiles can be easily generated using nanoimprinting.

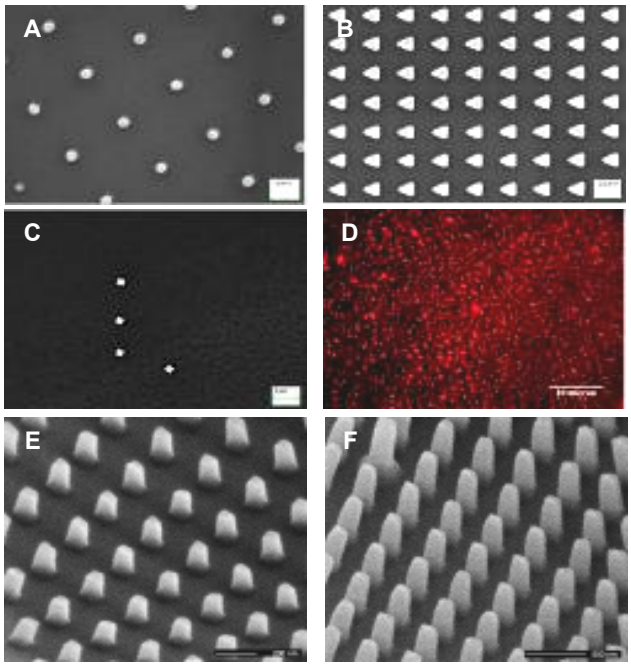

Figure 1: S-FIL Fabricated PEGDA nanoparticles: (A-C) SEM images (A) 50nm squares, 100nm scale bar; (B) 200nm triangles, 200nm scale bar; (C-D) Released nanoparticles (C) 400nm, 1μm scale bar, (D) Fluorescence image of 400nm particles with encapsulated fluorescently labeled goat anti-mouse IgG, 1μm scale bar; (E-F) SEM image at 52°D angle (E) 100nm squares, 200nm scale bar; (F) 200nm squares, 500nm scale bar [1]

In addition, particles of specific lateral dimensions, 50 nm (a), 200 nm (b), 400 nm (c, d) and 400 nm squares with ~500nm height (e), and 200nm squares with ~500nm height (f), were fabricated [1]. The heights of the features can be controlled by the drop pattern, volume of the macromer, the imprint force, and the etching depth of the template. **Figure 1 C-D** shows 400 nm square particles after release in water and 400 nm squares loaded with a fluorescently labeled antibody [1].

Conclusions: We have successfully generated uniform nanocarriers, as small as 50nm, having various shapes and aspect ratios. These particles are easily harvested from the silicon wafers using a biocompatible, one step release process. We also demonstrate efficient encapsulation and successful enzyme-triggered release of model drugs (proteins and nucleic acids) from these nanoparticles. The surfaces of these nanocarriers can be easily functionalized with cell targeting ligands. Further studies evaluating intracellular drug delivery and targeted drug release are currently underway.

References:

Luz Cristal Glangchai, Mary Caldorera-Moore, Li Shi, and Krishnendu Roy, Journal of Controlled Release, 2007. in press