High Throughput Nanoimprint Manufacturing of Shape-Specific, Stimuli-Responsive Polymeric Nanocarriers for Drug and Imaging Agent Delivery

PIs: Li Shi[1], S. V. Sreenivasan[1], Krish Roy[2], Dwayne LaBrake[3]

Graduate Students: Mary Caldorera-Moore[2], Patrick Jurney[1], Vikramjit Singh[1], Rachit Agarwal[2], Scott Marshall[1]

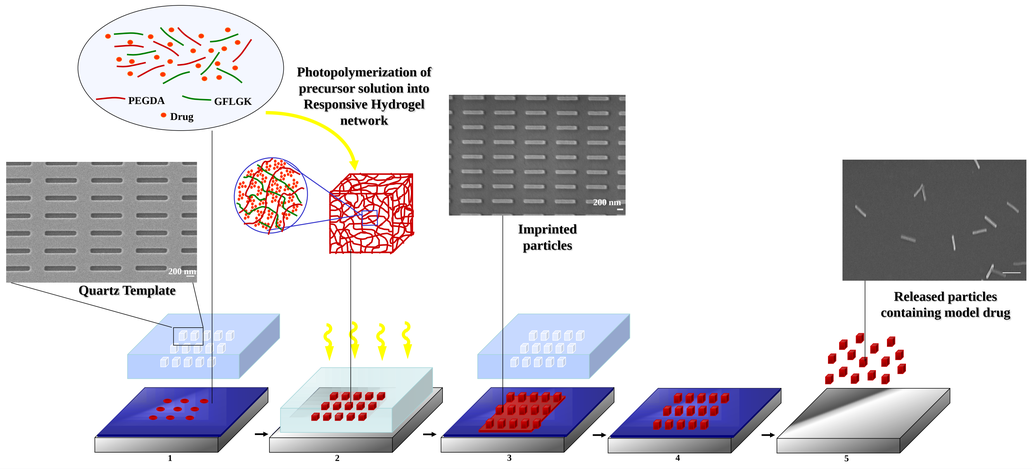
[1]Department of Mechanical Engineering, [2]Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX, [3]Molecular Imprint, Inc., Austin

Award # 0900715
Program: Nanomanufacturing, NIRT

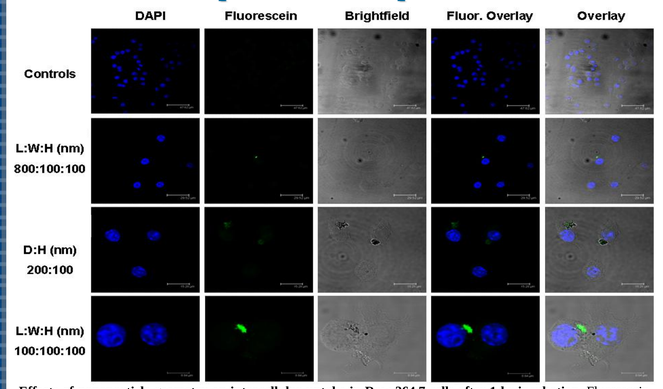
Abstract

A significant amount of research has been conducted on the development of hydrogel-based drug delivery systems that have the ability to swell or shrink in the presence of different environmental cues. However, despite significant progress, there remain critical limitations in synthesizing nanoparticles with highly controllable architecture (size, shape and aspect ratio) that can, at the same time, impart triggered release mechanisms. These parameters are essential for controlling *in-vivo* transport, bio-distribution, cellular uptake and drug release mechanisms. Recently, we have developed a nanofabrication technique using Jet and Flash Imprint lithography (J-FIL), to synthesize stimuli-responsive nanocarriers of precise sizes, shapes, and compositions. Our results indicate that hydrogel nanoparticles of a variety of shapes and aspect ratios can be fabricated at sub-50 nm dimensions. These shape-specific nanoparticles can also be disease-responsive through incorporation of enzymatically-degradable peptides in the particle matrix, providing release of encapsulated drugs or contrast agents in response to specific physiological or pathophysiological conditions. In order to verify that the specific shape of the nanocarriers is preserved during in-vivo transport in biofluids, experimental characterization and theoretical models have been carried out to determine the nanoscale swelling characteristics of such shape-specific nanoparticles. In addition, our *in-vitro* cellular uptake data indicates size-dependent internalization of the nanoparticles.

Summary of Fabrication Method:



Intracellular Uptake of Nanoparticles



Effects of nanoparticle geometry on intracellular uptake in Raw 264.7 cells after 1 hr incubation. Fluorescein containing particles (column 2) were introduced to Raw macrophage cells. Cell nuclei were stained with 6-Diamidino-2-phenylindole (DAPI) (column 1). Column 3 and 4 are overlay images illustrating localization of particles within cells in comparison to control cells (row 1).

Research Objectives:

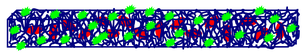
-Development of high a through-put, biocompatible nanoimprint technique

- Minimize or eliminate exposure of the imprinted nanocarriers to plasma etching, UV, or other chemicals
- Increase imprint throughout to >1 dose of drug loading in nanocarriers per hour

***-In vitro* characterization of fabricated hydrogel nanoparticles**

- Evaluation of encapsulation efficiency of therapeutic and contrast agents within hydrogel network
- Characterization of nanoscale hydrogel swelling behavior
- Evaluation of the effects of particle shape, size, and aspect ratio on intracellular uptake by cells

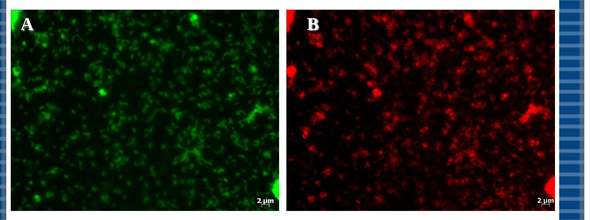
Dual Loaded Particles:



Flourescein tagged to the hydrogel network

Cy3 labeled siRNA encapsulated within the hydrogel network

- **Hydrogel network tagged with contrast agents for evaluation of particles *in vitro* and *in vivo* bio-distribution**
- **Fluorescently labeled siRNA (GAPDH) loaded within particles**



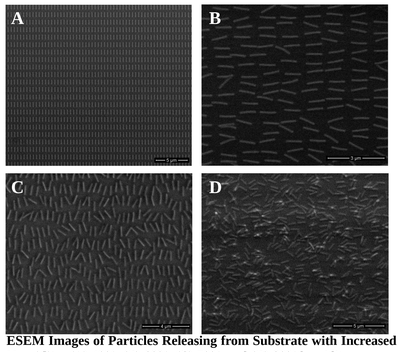
Fluorescent Microscopy of Dual Loaded Release Particles (A) FITC filter detecting flourescein-o-acrylate on hydrogel surface and (B) GAPDH-siRNA labeled with Cy3 encapsulated within the hydrogel network.

Characterization of PEG Nanoparticles:

METHOD 1: Environmental Scanning Electron Microscopy

1- Imaging with FEI ESEM

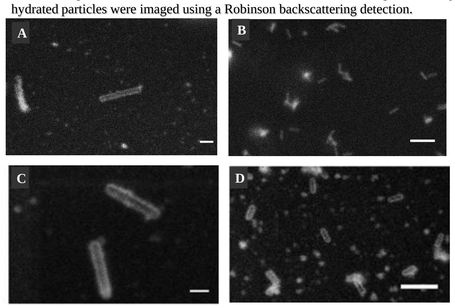
Fabricated particles on aqueous release layer were imaged in their native state under 2.0 Torr pressure. Moisture was pumped into the chamber to increase the chamber humidity leading to the release layer to dissolve releasing the particles from the surface. Even at 80% humidity particles` size do not significantly change.



ESEM Images of Particles Releasing from Substrate with Increased Humidity: (A) 40%, (B) 60%, (C) 70%, and (D) 80% humidity .

2- Imaging with QuantomiX capsules wet SEM

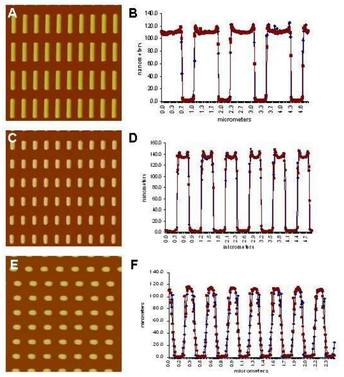
Fabricated particles were released into filtered DH_2O and incubated for 24 hours. Particle suspension was then added into the QuantomiX wet capsules. Fully hydrated particles were imaged using a Robinson backscattering detection.



ESEM Images of Released J-FIL Nanoparticles Using QuantomiX Wet Capsules. (A-B) 50 %(v) PEGDA 700 nanoparticles, (C-D) 33% (v) PEGDA 700 nanoparticles (A-C) 800 nm by 100 nm by 100 nm features and (B-D) 400 nm by 100 nm by 100 nm particles.

METHOD 2: Atomic Force Microscopy (AFM)

Fabricated particles adhered to the imprinting substrate were scanned in the dried state and swollen state using AFM to gain particle topography.

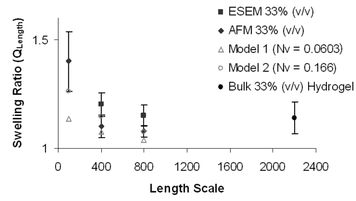


AFM scans of 33%(v/v) PEGDA 700 S-FIL fabricated particles: (A-B) 800 x 100 x 100 nm particles, (C-D) 400 x 100 x 100 nm particles and (E-F) 100 x 100 x 100 nm particles. (A, C, and E) scan tomography image, (A and C) 5 x 5 micrometer scan area, (E) 2.5 x 2.5 micrometer scan area, (B, D, and F) line scan of particle height profiles from AFM scan, where red is trace direction scan and blue is retrace direction scan.

Comparison of Results:

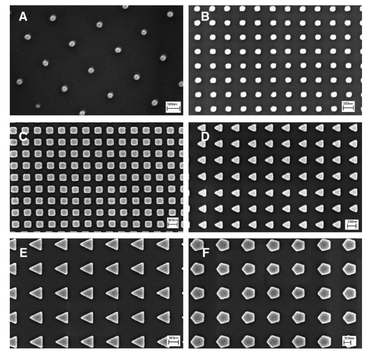
- The swelling ratio (Q) calculated from the length of the 800 x 100 x 100 nm and 400 x 100 x 100 nm particles from ESEM are comparable to the Q of bulk samples.
- Comparing the AFM and ESEM results: Q decreases due to the effect of substrate constraint in the AFM measurements.
- AFM results show that the Q decreases as the length of the constrained particles increases. This qualitatively agrees with the finite element calculations for the substrate-constrained particles.

Comparsion of Hydrogel Swelling of Nanoparticles Using Different Methodologies



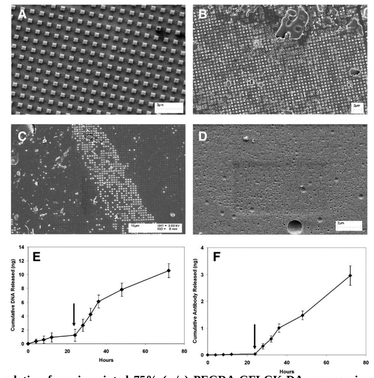
SEM of Different Shapes Fabricated

Particles of different shapes and sizes were fabricated using different templates patterned differently using EBL



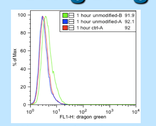
SEM images of J-FIL imprinted (100% w/v, MW 3400) PEGDA nanoparticles: (A) 50 nm squares (scale bar=100 nm), (B) 100 nm squares (scale bar=200 nm), (C) 200 nm squares (scale bar=300 nm), (D) 200 nm triangles (scale bar=200 nm), (E) 400 nm triangles (scale bar=300 nm), and (F) 400 nm pentagonal particles (scale bar=200 nm).

Environmentally Triggered Release Kinetics



Enzymatic degradation from imprinted 75% (w/v) PEGDA-GFLGK-DA nanocarriers (n=3): SEM images of control particles at 48 h in PBS: No Cathepsin B added (scale bar=2 μm) (B-D), Nanoparticles after 30 min, 12 h, and 48 h in Cathepsin B (25 U/mL) (scale bars=2 μm, 10 μm, and 2 μm) (D). Graphs showing stimuli-responsive release of biological agents encapsulated within imprinted PEGDAGFLGK-DA particles in response to 20 U/mL Cathepsin B over time: release profile of 0.16% (w/w) plasmid DNA encapsulated in 75% (w/v) PEGDA-GFLGK-DA nanoparticles (n=3) (E), and release profile of 0.075% (w/w) fluorescently labeled goat anti-mouse IgG encapsulated in 100% (w/v) PEGDA-GFLGK-DA nanoparticles (n=3) (F). Arrows indicate time points where Cathepsin B is added to the particles.

On-going and Future Work



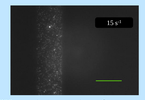
FACS histograms of control HEK 293 cells in compared to cells exposed to 100 nm fluorescein labeled nanoparticle

***In Vitro* Characterization:**

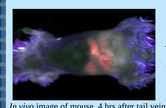
- Quantification of loading efficiency of various size particles
- Quantification of shape and size effects on particles internalization using fluorescent cell sorting (FACS)
- Characterization and optimization of carriers controlled drug release in cells

Characterization of Margination Dynamics

- Explore the effect of aspect ratio on margination and adhesion of nanoparticles
- Develop a particle dynamics model of non-spherical nanoparticle margination and adhesion dynamics



300 nm polystyrene spheres flown through a microfluidic channel of half-circle cross-section. Scale bar is 40 μm.



In vivo image of mouse, 4 hrs after tail vein injection of 800 nm x 100 nm x 100nm J-FIL particles containing fluorescein

***In Vivo* Characterization:** Bio-distribution of different shape and size J-FIL fabricated nanoparticles in mice.

Conclusions

We have demonstrated a nanoimprinting method for creating enzymatically-triggered nanocarriers of precise sizes and shapes for drug and contrast agent delivery. We have achieved particle size as small as 50 nm along with efficient stimuli-responsive release of encapsulated agents. The imprinted particles can be directly harvested into aqueous buffers using a simple, biocompatible process. We have conducted swelling studies on both bulk hydrogels and imprinted monodisperse hydrogel nanoparticles composed of various percent polymers 10-50% (v/v) PEGDA. Our measurement results show that the length swelling ratio of the nanoparticles is comparable to the bulk value when the length of the particle is longer than 400 nm while the width and height were 100 nm. While measurement of swelling ratio for sub-100 nm hydrogel particles remains a challenging characterization task, theoretical analysis of the hydrogel swelling behavior suggests that the highly crosslinked PEGDA MW 700 hydrogels do not swell significantly, and therefore the shape and size of these specific top-down fabricated nano-carriers can be preserved in aqueous environments for particle size larger than 100 nm. The material chemistry used here is also conducive of readily attaching specific ligands to the particle surface thus providing opportunities of cell targeted, disease-triggered delivery of drugs.

Our *in vitro* studies also qualitatively confirm that intracellular localization of nanoparticles is shape dependent. The smaller, cylindrical particles were more readily internalized by cells. The 800 nm x 100 nm x 100 nm particles were observed to be on the surface of the cells but not internalized, which suggest the particles are too large for endocytosis.

Acknowledgements

This work is supported in part by National Science Foundation award CMMI-0900715 and 0547409. MCM is an NSF graduate research fellow. The nanofabrication was conducted at the UT Austin Microelectronics Research Center (MRC), a member of the NNIN. The authors acknowledge technical assistance from the staff of MRC and Center for Nano and Molecular Science and Technology at UT Austin. ESEM images were conducted at FEI.