Microfluidic Platform with Hierarchical Micro/Nanostructures and SELEX Nucleic Acid Aptamer Coating for Isolation of Circulating Tumor Cells

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***Abstract*— Microfluidic platforms provide elegant schemes for isolation of rare cells from the blood. Hierarchical 3-dimensional architectures with silicon nanopillar forests draw advantage of unique surface interactions with target cells to aid capture and adhesion. Microchannels with numerically optimized designs of micro post arrays maximize contact frequency with the cells in flow. We describe a scheme to fabricate ordered nanopillar forests on the sidewalls of these micro posts and functionalize them with SELEX nucleic acid aptamers. Our microfluidic platform, designed for 90% contact probability, facilitates technologies for highly selective and specific capture of circulating tumor cells.**

I. INTRODUCTION

Circulating tumor cells (CTCs) can be detected in the blood stream of patients with epithelial cancers including those from breast, prostate, lung, and colon [1-5]. The ability to capture and characterize CTCs will provide a new methodology to determine the stage of a tumor and to guide the design of an appropriate therapeutic protocol [6, 7]. To this end, a lot of effort has been made in developing new technologies to isolate rare CTCs from human blood. The commonly used technologies mainly include immunomagnetic particles [8, 9] and microfluidic platforms [10-15]. However, CTCs in bloodstream always occur in extremely low quantities (1-10 tumor cells per billion red blood cells) that it is very challenging to isolate them with high yield, high efficiency, and high selectivity [10]. In fluid flow conditions, hydrodynamic resistance between a cell and a solid affinity surface can drive the cell away from the surface before the cell has the chance to bind to the surface [10, 16, 17]. In addition, shear stress developed in the fluid flow can cause detachment of a captured cell. These factors could lead to low capturing efficiency [10]. Furthermore, recovering positively captured cells in an unperturbed state is still a challenge when anti-EpCAM is used as the immobilized complementary ligand [10, 18, 19]. In this paper, we present the use of novel multiscale 3-dimensional structures coated with nucleic acid aptamers generated by in vitro cell-SELEX in a microfluidic platform for CTC isolation applications, which can help to solve the above mentioned challenges.

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II. EXPERIMENTAL

A. Microcrofluidic Platform Design

As shown in Fig. 1, a micro post array with core/shell structures is fabricated in a microchannel. The microstructure includes a solid core and a porous shell decorated with a silicon nanopillar (SiNP) forest. The microfluidic system with posts generates optimal flow characteristics and can increase the contact frequency and duration of interaction between rare cells and capturing elements. Nano-structures allow enhanced local topographic interactions with nanoscale components of the cellular surface (microvilli and filopodia on CTC surface), which are of comparable length scale as the nanopillars. The interactions could result in improved cell-capture affinity when compared to unstructured (i.e., flat silicon surface) substrates [19] and the smooth red blood cell surface. Compared to the flat surface, the nanopillar forest on the surface of micro posts would allow for reduced hydrodynamic resistance [17]. Nucleic acid aptamers are conjugated to the surfaces of micro posts for improved cell capture. These aptamers have numerous merits for biological and biomedical applications, by virtue of their high binding affinity, specificity and robustness. Furthermore, their small size, tunable binding kinetics, ease of synthesis and little immunogenicity or toxicity are advantageous for providing capture functionality [20-22].

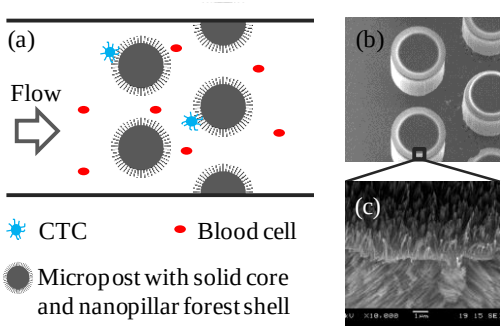

Figure 1. (a) Schematic of CTC isolation in a microchannel with micro post arrays. The micro post includes a solid core and a nanopillar forest shell. (b) A SEM image showing the micro post array with core/shell structure in a microchannel. (c) A SEM image showing the nanopillars on the surface of a micro post.

B. Microfabrication

The fabrication processes are shown in Fig. 2. The microchannel and the micro posts were defined on a <100> silicon wafer with 1 µm thick low stress silicon nitride using a two-step deep reactive ion etching (DRIE) process. Aluminum was used as an additional DRIE protective mask. First, isotropic SF_6 plasma was used to transfer pattern to the silicon nitride, with minimal undercut into the underlying silicon substrate (Fig. 2a). Next, the Bosch™ process was used to create the micro posts (Fig. 2b). Prior to growing nanostructures on the surface of micro posts, the photoresist and aluminum were removed, and polymer residues from the DRIE process were subjected to Piranha cleaning and ashing in oxygen plasma (Fig. 2c). Surface quality being critical for nanopillar synthesis, a short oxidation step in a tube furnace at 1100^0C was employed in order to treat the scallop sidewall roughness on micro posts (Fig. 2d). Subsequently, the oxide was etched, and the silicon nitride was to serve as a protective mask for the top surfaces during nanopillar fabrication (Fig. 2e). SiNPs were synthesized on the surface of the micro posts and microchannel walls by an aqueous electroless metal assisted chemical etching (MACEtch) method using Ag+/HF solutions (Fig. 2f). The surface of the micro posts in the microchannel was functionalized with aptamers (Fig. 2g) and capped for experiments (Fig. 2f). Aptamer binding on silicon dioxide surface was carried out by a well known "click chemistry", as shown in the schematic in Fig. 3.

Previously, MACEtching has been demonstrated using a variety of oxidizers and etchants for flat substrates [23]. In our work, we have developed a new technique to etch SiNPs into the sidewalls of DRIE patterned surfaces, which facilitates designing truly hierarchical micro/nanostructures and nanoscale patterning along preferred crystal directions. Fig. 4 shows SiNPs that have been etched into the sidewalls of test structures comprised of circular and triangular micro posts that are ca. 50 µm deep. In both cases, the micro posts demonstrate complete SiNP coverage and the evolution of the nanopillars is along the <100> crystal direction as seen by the tangential planes to the circular post and intersections of edges for the triangular post.

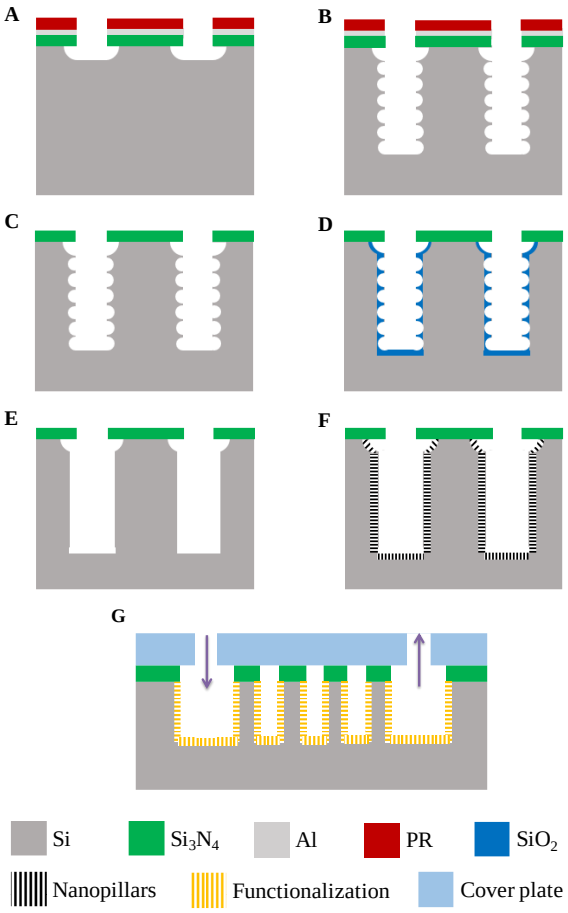

Figure 2. Schematic of MEMS fabrication process

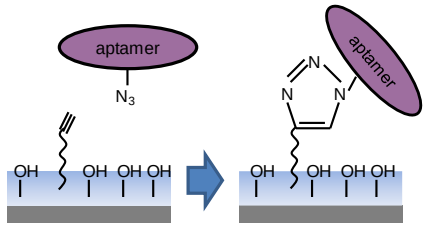

Figure 3. Schematic of the click chemistry process for functionalizing surfaces with aptamers.

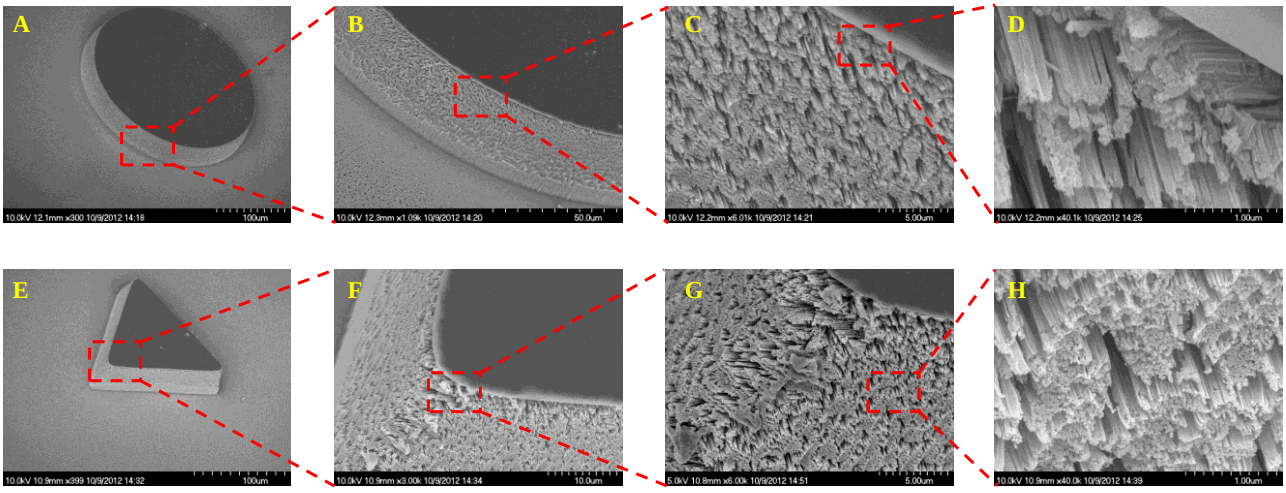

Figure 4. SEM images of MACEtched SiNPs fabricated on sidewall surfaces of (a – d) circular and (e - h) triangular micro posts along <100> planes.

C. Cell Capturing Experiment

We have conducted a preliminary study on a glass slide for cell capturing. As shown in Fig. 5, the naked glass surface could induce significant nonspecific cell attachment. When the glass surface was functionalized with polymers, nonspecific cell binding was barely observed. It indicates that the polymer coating is very efficient at eliminating non-specific cell binding. More importantly, after chemical conjugation of cell type-specific aptamers to the polymer coating, target cells could be effectively captured on the device surface, and demonstrates the utility of the approach towards capture of rare cells in a dynamic environment.

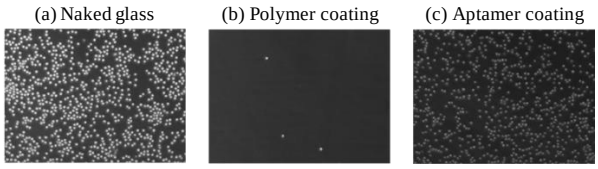

Figure 5. Preliminary study of cell capture. (a) no aptamer; (b) polymer coating; (c) with aptamer.

III. Modeling and Simulation

Optimal device design was synthesized using Finite Element Modeling. Comsol Mutliphysics v4.2a was used to create 2D streamline models and carry out simulations for flow through micro posts consisting of a solid inner core and an outer porous shell. Brinkman's equations were used for flow in the porous region and Navier-Stokes equations were used for the free media flow region. This coupled model for porous and free media flow has been previously used in literature [17, 24-26]. For our model, we have considered a constant permeability of 1 x 10^{-11} m^2 and 70% porosity for the porous shell of the micro post. The fluid viscosity was taken to be as that of water. An inlet velocity of 0.5 mm s^{-1} was maintained for simulation purposes. Since circulating tumor cells have a relatively larger diameter as compared to that of normal blood cells, an average particle size of 18 µm was assumed as the particle diameter [27]. A constant post diameter of 100 µm was assumed. The core diameter and porous shell diameter were then varied in order to achieve highest capture efficiency.

The posts shown in Fig. 6 were arranged in an equilateral triangle manner. But every post in the (4n)th column will have an upward shift by 15 µm and every post in the (4n+2)th column will have a downward shift by 15 µm. "n" here is the column number in the arrangement. This type of arrangement has been proved to have good capture efficiency in previous studies. The posts are equally spaced out at a distance of 50 µm from each other. The condition used for capturing particles was that if the distance between the post surface and the center of particle is less than that of the particle radius, then the particle is captured. To optimize the capture efficiency, the ratio of solid core diameter to porous shell diameter was compared. Fig. 6 shows the simulation results of particle capture with this arrangement with a solid core diameter of 80 µm.

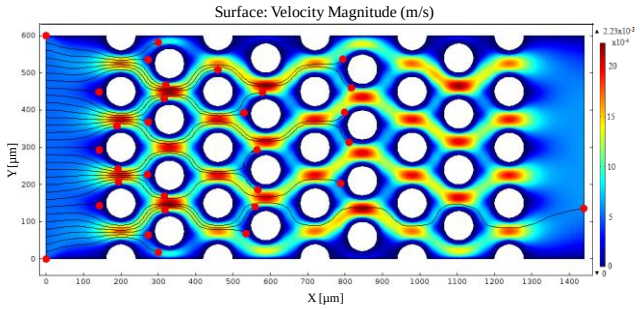

Figure 6. Simulation result of particle capturing in a microfluidic device with an array of core/shell micro posts. The micro post has a solid core diameter of 80 µm and an outer porous shell of 20 µm. The color represents the velocity fields.

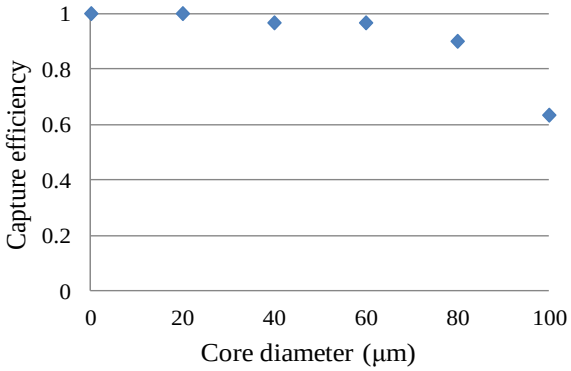

Figure 7. Variation of capture efficiency with increasing core diameter. 100% capture efficiency is achieved when the solid core is absent and the post is fully porous. Lowest capture efficiency is 65% for 100% solid post.

Capture efficiency was defined as the ratio of the number of particles captured on the surface of microposts to the number of particles released. Simulations were performed to calculate the capture efficiency while varying the solid core diameter. Fig. 7 illustrates the variation of capture efficiency with the solid core diameter. It was found that for a fully porous post without a solid inner core, the efficiency was 100%. However, keeping the fabrication constraints in mind, a solid core with a diameter of 80 µm yielded 90% capture efficiency. The fully solid posts only have 65% efficiency.

IV. Conclusion

We have developed a novel microfluidic platform for in-vitro capture of CTCs. Numerical modeling and simulation have been carried out to optimize designs of micro posts array in a microchannel. Nanoporous architecture on the sidewall of micro posts, implemented by our schemes to fabricate SiNP forests, allows interactions with nanoscale features on CTCs to increase residence times and facilitates cell capture. By combining the hierarchical micro/nanoscale architecture with robust nucleic acid aptamers, the microfluidic platform can achieve CTC capture with high specificity and selectivity, paving the way for improved diagnosis and detection of cancers.

ACKNOWLEDGMENT

This work is supported by the Research Support Funds Grant (RSFG) from Indiana University Purdue University Indianapolis and U.S. NSF grant (1033212/1340173). Micro-Nano-Mechanical Systems Laboratory at the University of Illinois at Urbana-Champaign provided the use of microfabrication facilities. SEM imaging was carried out in the Frederick Seitz Materials Research Laboratory Central Facilities, University of Illinois.

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