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Biodegradable nano-films for capture and non-invasive release of circulating tumor cells

Wei Li [a, b, 1], Eduardo Reátegui [c, d, 1], Myoung-Hwan Park [a], Steven Castleberry [a], Jason Z. Deng [a, e], Bryan Hsu [a], Sarah Mayner [a], Anne E. Jensen [d], Lecia V. Sequist [f, h], Shyamala Maheswaran [c, f], Daniel A. Haber [f, g, h], Mehmet Toner [c, d], Shannon L. Stott [d, f, h, **], Paula T. Hammond [a, e, *]

[a] Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA
[b] Department of Chemical Engineering, Texas Tech University, Lubbock, TX, USA
[c] Department of Surgery, Harvard Medical School, Boston, MA, USA
[d] Center for Engineering in Medicine, Harvard Medical School, Boston, MA, USA
[e] David H. Koch Institute of Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, USA
[f] Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA
[g] Howard Hughes Medical Institute, Chevy Chase, MD, USA
[h] Department of Medicine, Harvard Medical School, Boston, MA, USA

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ABSTRACT

Selective isolation and purification of circulating tumor cells (CTCs) from whole blood is an important capability for both clinical medicine and biological research. Current techniques to perform this task place the isolated cells under excessive stresses that reduce cell viability, and potentially induce phenotype change, therefore losing valuable information about the isolated cells. We present a biodegradable nano-film coating on the surface of a microfluidic chip, which can be used to effectively capture as well as non-invasively release cancer cell lines such as PC-3, LNCaP, DU 145, H1650 and H1975. We have applied layer-by-layer (LbL) assembly to create a library of ultrathin coatings using a broad range of materials through complementary interactions. By developing an LbL nano-film coating with an affinity-based cell-capture surface that is capable of selectively isolating cancer cells from whole blood, and that can be rapidly degraded on command, we are able to gently isolate cancer cells and recover them without compromising cell viability or proliferative potential. Our approach has the capability to overcome practical hurdles and provide viable cancer cells for downstream analyses, such as live cell imaging, single cell genomics, and *in vitro* cell culture of recovered cells. Furthermore, CTCs from cancer patients were also captured, identified, and successfully released using the LbL-modified microchips.

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1. Introduction

Circulating tumor cells (CTCs) present in the bloodstream of patients with cancer, originate from primary or metastatic tumor sites, and are thought to mediate the hematogenous spread of cancer to distant sites [1–4]. Technological hurdles have limited their isolation and characterization because these cells are extremely rare (1 in 10^9 blood cells) and mixed with normal blood components [5]. Isolation of CTCs is of great interest in the scientific community due to their usefulness in analyzing the diagnosis and treatment of patients with epithelial cancers in lieu of invasive biopsies. In order to capture CTCs from the bloodstream, multiple isolation approaches have been discovered thus far, which in general, take advantage of differences in physical cell properties or known cell surface markers [4–10].

Methods for CTC capture and release can be separated into macro- and micro-processes. The former group includes density gradient centrifugation [11], microfiltration [12–14], and use of

* Corresponding author. Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA.
** Corresponding author. Center for Engineering in Medicine, Harvard Medical School, Boston, MA, USA.
E-mail addresses: sstott@mgh.harvard.edu (S.L. Stott), hammond@mit.edu (P.T. Hammond).
[1] W. L. and E. R. contributed equally to this work.

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antibody-modified magnetic beads [15–18]. Examples of the latter include use of lectin-modified microposts [19–21], DNA aptamers attached to silicon nanowires [22–24], antibody-modified photolabile linkers on glass substrates [25,26], APBA-functionalized multi-walled carbon nanotube films [27,28], interaction between calmodulin with a calmodulin-binding peptide in the presence of calcium, and cryogels [29]. Despite these many approaches, there remain several issues with the release processes that must be addressed to realize the full potential of CTCs as a diagnostic and research tool. First, the cells must be viable; unfortunately, many existing methods exhibit lower than acceptable yields in the release of unharmed living cells. Second, the phenotype of the cells must be preserved in order to accurately study the cells. Stresses from shear force, non-physiologic temperature variation, aggressive reagents such as trypsin and UV exposure are known to affect the phenotype of captured cells [6,30,31]. In addition, the method must achieve both high cell recovery as well as high cell purity. Finally, the method must be feasible for disposable point-of-care use even in remote areas; it must not require excessive lab equipment, or be limited to electrical and optical means of cell detachment.

We present a new method of capture and release of CTCs using a microfluidic device, the ^{HB}CTC-chip [1,3], modified with enzymatically degradable nano-films that are conjugated with antibodies to a variety of specific cell surface markers. We show that layer-by-layer (LbL) [32–35] assembly as an effective method to coat nanometer scale film inside microfluidic devices with complex microstructures. We achieved 80% capture efficiency and 95% release efficiency for spiked prostate cancer cells with heterogeneous levels of expression of the surface antigen EpCAM, as well as CTCs in the blood samples of patients with metastatic lung cancer. The viability of released cells was demonstrated to be ~90%. For the patient samples tested, CTCs were detected, captured and successfully released using the biomaterial coated-microchip we developed. The CTCs in the patient blood samples were found to range from 3.4 to 4.9 CTCs/mL, while less than 0.5 CTCs/mL was found in control samples.

2. Materials and methods

2.1. Materials

Alginate (ALG) (Pronova UPMVG, 60% guluronate, 40% mannuronate, M_w = 120 k and 280 k) was purchased from Novamatrix, Norway. Hyaluronic acid (HA, M_w = 200 k), poly(allylamine hydrochloride) (PAH, M_w = 60 k), poly-L-lysine (PLL, M_w = 50 k to 70 k), low molecular weight chitosan (LMWC, M_w = 15 k), diethylaminoethyl dextran (DEAED, M_w = 500 k) and all other reagents were purchased from Sigma Aldrich, USA.

2.2. Fabrication of herringbone CTC chip (^{HB}CTC-chip)

Negative photoresist (SU-8, MicroChem) was photolithographically patterned on silicon wafers to create masters with two-layer features [3]. The first layer is the main microfluidic channel and the second layer forms the herringbone structures. The heights of SU-8 features are ranging from 25 to 75 μm on the masters. Polydimethylsiloxane (PDMS, SYLGARD 184, Dow Corning) was poured, degassed, and cured in a conventional oven at 75 °C for 24 h. The cured PDMS replicas were removed from the molds, oxygen plasma treated, and bonded to glass substrates to form the final devices.

2.3. Biotin modification of ALG and HA

Alginate and hyaluronic acid were modified with biotin hydrazide (Sigma B7639) using standard carbodiimide reaction [36]. Briefly, 1.0 wt% of ALG or HA solution was prepared in MES buffer, pH = 6.0. Per 50 mL of ALG or HA solution, 80 mg of biotin hydrazide, 360 mg of 1-ethyl-3-[3- dimethylaminopropyl] carbodiimide hydrochloride (EDC, Pierce 22980), and 204 mg of hydroxysulfosuccinimide (Sulfo-NHS, Pierce 24510) were added and reacted for 3 h, after which time the solution was dialyzed against deionized H_2O for 48 h and lyophilized. Alginate or hyaluronic acid was reconstituted at 2 mg/mL in deionized H_2O prior to use.

2.4. Preparation of nano-films

Lay-by-Layer (LbL) assembly of charged polymers were applied to build nanofilms inside microfluidic devices [37]. Biotin modified ALG and HA were used to prepare anionic polymer solution, while PAH, PLL, LWMC and DEAED were used to prepare cationic polymer solutions. The initial experiments were performed using a simplified microfluidic device comprising a straight PDMS microchannel with the dimension of 400 μm (width) × 100 μm (height) × 10 mm (length) sealed on a glass substrate. Briefly, glass substrate was treated with oxygen plasma for 5 min and immediately bonded to oxygen plasma treated PDMS replicas to form final devices. For LbL assembly of nano-films, a cationic polymer solution (2 mg/mL) was first injected into the inlet of the device to occupy all the inside area, sit for 5 min for the absorption of polymers, then the solution was removed by air flow and the device was washed with 1 mL DI water for two times, then subsequent anionic polymer solution (2 mg/mL) was injected into the device and allowed a 5 min absorption time, after which the device was washed with DI water. This process was repeated 5 times at room temperature under sterile conditions.

2.5. Degradation of nano-films

To visualize the degradation of nano-films, a solution of 0.05 mg/mL Streptavidin Dylight 650 fluorescent conjugates (Thermo Scientific) in PBS was introduced into the modified microchannels and stored at 4 °C for 4 h. Then the avidin solutions were removed and the devices were washed thoroughly with DI water. The fluorescent intensity of each film was recorded using a fluorescent-optical microscope (BX53, Olympus) at the same exposure time of 2 s. A 2 mg/mL enzyme solution (alginate layse or hyaluronate lyase in PBS containing 1 wt% bovine serum albumin, BSA) was introduced into the microchannel and kept flowing for 30 min at 2.5 mL/h. After thoroughly washing the microchannel with DI water, the fluorescent intensity of the film was recoded using the same exposure time of 2 s. All the images were analyzed using ImageJ (NIH), and the fluorescent intensities of different types of films were normalized to the maximum intensity, which was obtained from ALG/LMWC film before degradation. The change (%) of FI before and after degradation was calculated by comparing the change of normalized value of FI over the initial FI for all the film compositions.

2.6. Optimization of the degradation of ALG/PAH nano-film

A series of ALG/PAH nano-films were made using the method described above. Four types of 2 mg/mL ALG solutions were prepared as following: ALG with molecular weight of 132 k at pH 4.5 and pH 7.2, as well as ALG with molecular weight of 280 k at pH 4.5 and 7.2. PAH used in this experiment was labeled with fluorescein. Film thickness was measured using an optical surface profilometer (Dektak 150, Veeco). Fluorescent intensities of four types of films were recorded at t = 0, 10 min, 20 min, and 30 min. The degradation efficiency (%) was calculated by comparing the change of FI over the