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Adhesion-based tumor cell capture using nanotopography

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ABSTRACT

Circulating tumor cells (CTCs) shed from primary tumors, transport through the blood stream to distant sites, and cause 90% of cancer deaths. Although different techniques have been developed to isolate CTCs for cancer detection, diagnosis and treatment, the heterogeneity of expression of the target antigen and the significant size variance in CTCs limit clinical applications of antibody- and size-based isolation techniques. Cell adhesion using nanotopography has been suggested as a promising approach to isolate CTCs independent of surface marker expression or size of CTCs. However, the nanotopographies studied are mainly nanopillars; the influence of other nanotopography such as nanogratings and their dimensions on tumor cell capture remains to be investigated. This study examined capture performance of several cancer cell lines of different types, surface marker expression and metastatic status on nanotopographies of various geometries and dimensions without antibody conjugation. The cancer cells exhibited differential capture performance on the nanotopographies with an efficiency up to 52%. Compared with flat surfaces and isotropic, discrete nanopillars, nanogratings favored cancer cell adhesion, thus improving the capture efficiency. The influence of nanotopography height studied, on the other hand, was less significant. This study provides useful information to optimize nanotopography for further improvement of CTC capture efficiency.

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1. Introduction

Cancer metastasis, initiated by circulating tumor cells (CTCs) migration from primary tumors through the blood stream to distant sites of the body, causes 90% of cancer deaths [1]. Although the cellular and molecular characterization of CTCs holds great promise for cancer detection, diagnosis and treatment, realization of this potential remains limited by current challenges associated with CTC isolation techniques [2]. The major hurdles in advancing CTC isolation techniques include rarity and heterogeneity of CTCs. CTCs are extremely rare, as few as one CTC per 10^9 normal blood cells in the blood of patients with metastatic cancer [3]. CTCs also display heterogeneity in expression of target antigens and variation in cell size. Nevertheless, different techniques have been developed to capture CTCs.

The most widely used CTC capture techniques rely on positive selection based on surface biomarkers such as epithelial cell adhesion molecule (EpCAM) expression on tumor cells [4]. For instance, CellSearch assay, the most standardized technology, uses ferrofluids loaded with an EpCAM antibody (anti-EpCAM) to capture CTCs through a magnetic field. The advent of microfluidic technology advances the CTC capture techniques. CTC capture efficiency has been improved by enhancing CTC-antibody interactions via optimizing the microchannel dimensions [5], introducing microscale pillars [6], and generating microvortices [7]. Although EpCAM is expressed in the cells of epithelial origin, the EpCAM expression on tumor cells varies with tumor type [8], some cells even express no EpCAM [9]. Additionally, invasive tumor cells tend to lose their epithelial antigens via the epithelial-to-mesenchymal transition process [10]. The antibody-based capture techniques are thereby limited to the tumor cells expressing the specific antigen [11–13]. Because the CellSearch assay has relatively low sensitivity on some tumor cells expressing low or no EpCAM, the assay only achieved a median yield of approximately one CTC per milliliter and typically low purity [14,15]. To overcome the limitation of heterogeneous surface marker expression, several physical properties distinguishing CTCs from most normal blood cells have been utilized to capture CTCs. The properties include size, density, charge, motility, and some properties of specific cell types (e.g., melanocytic granules in melanoma cells) [16–18]. For instance, because most epithelial cells have a larger size than normal blood cells, the size-based microsieve and microfiltration device have been developed to isolate breast, gastric, and colon tumor cell lines including EpCAM-negative tumor cells [17,19–21]. However, CTCs are not always larger than leuko-

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cytes. For example, monocytes have a similar size (15–25 μm) to CTCs [22]. This similarity in size could largely decrease the capture sensitivity and purity, thus impeding the clinical applications of size-based capture techniques [23].

Emerging compelling evidence continues to show that substrate nanotopography has profound influence on cell adhesion, suggesting cell adhesion as a promising approach to CTC capture. Inspired by nanostructured surface (e.g., microvilli, microridges and cilia) of cancer cells [24] and enhanced cancer cell-nanotopography interactions [25], silicon nanowires [26,27], polystyrene (PS) nanotubes [28] and hierarchical nanowire arrays [29] have been fabricated and conjugated with anti-EpCAM to significantly improve sensitivity and efficiency of CTC capture. Strikingly, by taking advantage of the differential adhesion preference of cancer cells to nanotopography compared with normal blood cells, Chen et al. demonstrated high selectivity and high efficiency of CTC capture by using reactive ion etching (RIE)-generated nanorough glass surfaces regardless of the surface marker expression or physical size of the CTCs [30]. Current studies were mainly based on nanopillars. Although the effects of nanopillar diameter and spacing on tumor cell capture were investigated in details [31], other typical nanotopographies such as nanogratings need to be explored. Previously, we observed that nanogratings provided continuous contact guidance to human lung fibroblast cells while discrete, isotropic nanopillars tended to disrupt the formation and growth of focal adhesions, suggesting that nanotopography geometry had a significant influence on cell adhesion [32]. To successfully translate adhesion-based CTC capture technique using nanotopography to clinical settings, it is highly desirable to understand how tumor cells interact with nanotopographies of various geometries and dimensions.

Herein, we investigated capture performance of four human cancer cell lines on representative nanotopographies. The nanotopographies of various geometries (nanoscale gratings and pillars) and dimensions (feature size, spacing and height) were engineered on elastomeric polydimethylsiloxane (PDMS) substrates. The cancer cell lines were MCF7 (a luminal non-metastatic breast cancer cell line), A549 (an adenocarcinomic alveolar basal epithelial cell line), HeLa (a cervical cancer cell line) and MDA-MB-231 (a basal aggressive metastatic breast cancer cell line). Thereby, we were able to examine the influence of geometry and dimensions of nanotopography on cell adhesion and capture performance of the cancer cells of different cell types, surface marker expression (EpCAM positive MCF7 and A549 cells vs EpCAM negative HeLa cells and MDA-MB-231), and metastatic status (non-metastatic MCF7 vs metastatic MDA-MB-231). This study helped our understanding of nanotopography enhanced CTC capture through tumor cell-nanotopography interactions.

2. Experimental section

2.1. Cell culture

All cancer cell lines were purchased from American Type Culture Collection (ATCC; Manassas, VA). MCF7 were cultured in Eagle's Minimum Essential Medium (EMEM; ATCC) supplemented with 10% (v/v) fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA), 1% (v/v) penicillin/streptomycin (Life Technologies, Carlsbad, CA) and 0.01 mg/ml human recombinant insulin (Sigma-Aldrich, St Louis, MO). MDA-MB-231 were cultured in Minimum Essential Media (MEM; Thermo Fisher Scientific) supplemented with 10% (v/v) FBS, 1% (v/v) L-glutamine (Life Technologies) and 1% (v/v) penicillin/streptomycin. HeLa and A549 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich) supplemented with 10% (v/v) FBS, 1% (v/v) L-glutamine 1% (v/v) penicillin/streptomycin. The cultures were maintained at 37° C in a humidified atmosphere containing 5% CO_2. The culture media was replaced every 3 days.

2.2. Preparation of nanotopographies

Nanotopography was generated by using electron beam lithography (EBL) and replicated into PDMS substrate as previously described [32]. Briefly, the nanopattern was designed and written onto a poly(methylmethacrylate) (PMMA) thin film on a silicon substrate by using EBL and then etched in the silicon substrate by applying RIE process. The generated nanotopography was cast with a mixture of PDMS resin and curing agent (Sylgard 184 silicone elastomer kit; Corning, Corning, NY) at a 10:1.05 w/w ratio, followed by curing at 70 °C for 4 h. The PDMS nanotopography was expanded to a large area by applying a stitch technique [33]. The stitched mold was then imprinted into PS substrates to make a PS master mold, from which working PDMS nanotopographies were produced.

The working PDMS nanotopographies were punched to discs fitting in the wells of a 48-well plate. The discs were oxygen plasma treated at 300 mT, 50 W for 30 s in a PX-250 Plasma Asher (Nordson MARCH, Concord, CA) to render a hydrophilic surface. The PDMS nanotopographies and flat surfaces as control were sterilized in 70% ethanol followed by UV exposure, each for 30 min, and then incubated with 5 μg/cm^2 collagen I (Corning) for 1 h prior to cell seeding.

2.3. Cancer cell capture

The cancer cells were first labeled with either CellTracker Green or CellTracker Red (Life Technologies) according to the manufacturer's instruction. Briefly, the cells were incubated in 5 mM CellTracker in DMEM solution (serum free) at 37° C for 30 min. The cells were then trypsinized using 0.25% trypsin-EDTA (Sigma-Aldrich) after phosphate buffer saline (PBS; Sigma-Aldrich) rinse, resuspended in 1 mL fresh media, and diluted to a desired seeding density of 500 cells/well. The cell suspension was added onto the PDMS discs in 48-well plates and incubated for a predefined capture time (1 h, 2 h or 4 h).

To achieve accurate cell counting, the whole PDMS surface with the cells was first scanned by using a Nikon Swept Field microscope with 10× objective (Nikon, Melville, NY) at the predefined time point. Multiple images from the scanned field of 1 cm x 1.03 cm rectangle were stitched to a single image using Nikon NIS-Element software, covering the whole well of 48-well plates. Subsequently, the culture media was carefully aspirated and the sample was gently rinsed with PBS to remove any non-adhered cells. The PDMS disc with captured cells was scanned again to count the captured cells by repeating the aforementioned scanning process.

ImageJ (http://imagej.nih.gov/ij/) was applied to quantify the numbers of seeded cells and captured cells by using the "analyze particle" function. The cells were highlighted by adjusting the threshold and the image was converted to a binary image prior to analyzing the particles.

2.4. Cell area measurement

MCF7 cells were pre-labeled with CellTracker Red and seeded at a density of 1000 cells/cm^2 on the PDMS substrates including the nanotopographies and flat surfaces. After 4 h cultivation, the cells were fixed using 4% paraformaldehyde (PFA; Electron Microscopy Sciences, Hatfield, PA) in PBS for 30 min, and mounted on a coverglass using proLong Gold Antifade Reagent with 4,6-diamidino-2-phenylindole (DAPI; Life Technologies) overnight at room temperature. At least 50 cells were imaged using a Nikon Swept Field microscope with 40 x oil objective. Cell area was quantified using ImageJ software. Firstly, the cells were highlighted by

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