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Surfactant functionalization induces robust, differential adhesion of tumor cells and blood cells to charged nanotube-coated biomaterials under flow

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ABSTRACT

The metastatic spread of cancer cells from the primary tumor to distant sites leads to a poor prognosis in cancers originating from multiple organs. Increasing evidence has linked selectin-based adhesion between circulating tumor cells (CTCs) and endothelial cells of the microvasculature to metastatic dissemination, in a manner similar to leukocyte adhesion during inflammation. Functionalized biomaterial surfaces hold promise as a diagnostic tool to separate CTCs and potentially treat metastasis, utilizing antibody and selectin-mediated interactions for cell capture under flow. However, capture at high purity levels is challenged by the fact that CTCs and leukocytes both possess selectin ligands. Here, a straightforward technique to functionalize and alter the charge of naturally occurring halloysite nanotubes using surfactants is reported to induce robust, differential adhesion of tumor cells and blood cells to nanotube-coated surfaces under flow. Negatively charged sodium dodecanoate-functionalized nanotubes simultaneously enhanced tumor cell capture while negating leukocyte adhesion, both in the presence and absence of adhesion proteins, and can be utilized to isolate circulating tumor cells regardless of biomarker expression. Conversely, diminishing nanotube charge via functionalization with decyltrimethylammonium bromide both abolished tumor cell capture while promoting leukocyte adhesion.

1. Introduction

Metastasis, the spread of cancer cells from a primary tumor to anatomically distant organs, contributes to over 90% of cancer-related deaths [1]. Cancer cells shed from the primary tumor, which can number as many as one million cells per gram of tumor per day [2,3], enter the bloodstream as circulating tumor cells (CTCs) via the process of intravasation [4,5]. Once in blood, CTCs must withstand fluid shear forces and immunological stress to translocate to microvessels in anatomically distant organs [6,7]. CTCs adhesively interact with receptors on the endothelial cell wall under flow, in a manner similar to the leukocyte adhesion cascade involved in inflammation and lymphocyte homing to lymphatic tissues [8,9]. Recent work has shown that CTCs display glycosylated ligands similar to leukocytes, which can trigger the initial adhesion with selectin receptors on the endothelium [10,11]. Due to their rapid, force-dependent binding kinetics, selectins can initiate CTC rolling adhesion along the blood vessel wall [12,13]. CTCs transition from rolling to eventual firm adhesion, allowing for transmigration into tissues and the formation of secondary tumors [14]. While surgery, radiation, and chemotherapy have proven generally successful at treating primary tumors that do not invade the basement membrane, the difficulty of detecting distant micrometastases has made the majority of metastatic cancer treatments ineffective. As a means to combat metastasis, approaches are currently being explored to target and kill CTCs in the bloodstream before the formation of secondary tumors [15–18]. Additionally, methods are being developed to isolate CTCs at high purity levels from patient blood, for the development of personalized medicine regimens for those with metastatic cancer [19–21].

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CTCs are sparsely distributed in the bloodstream, with CTC concentrations as low as 1–100 cells/mL [22]. Their separation and isolation from blood is commonly referred to as a "needle in a haystack problem", as leukocytes and erythrocytes are present in concentrations of one million and one billion cells per milliliter of blood, respectively [7,23]. Thus, numerous techniques for CTC isolation are in development, including magnetic bead-based CTC separation assays [24], flow-based microfluidic platforms for rapid isolation of CTCs from whole blood [25,26], and devices to separate tumor cells based on their chemotactic phenotype [27]. Our lab has recently developed microscale flow devices that mimic the metastatic adhesion cascade process to capture and separate CTCs from whole blood under flow conditions. Utilizing surfaces coated with recombinant human E-selectin (ES) and antibodies against the CTC markers epithelial cell adhesion molecule (EpCAM) and prostate-specific membrane antigen (PSMA), we have fabricated flow devices to rapidly separate viable CTCs from patient blood and deliver targeted chemotherapeutics to cancer cells [26,28]. However, improvement of current CTC isolation purity levels is challenged by the fact that both CTCs and leukocytes express ES ligands [8].

Halloysite nanotubes (HNT) are naturally occurring clay minerals that are typically 800 ± 300 nm in length, 50–70 nm in outer diameter, and 10–30 nm in inner diameter [29]. Halloysite ($Al_2Si_2O_5(OH)_4$) is a two-layered (1:1) aluminosilicate that consists of an external siloxane (Si–O–Si) surface and an internal aluminol (Al–OH) surface [30]. At physiological pH, HNT has a negatively charged outer surface and a positively charged inner lumen [31], which has been exploited for the encapsulation and sustained release of drugs such as Nifedipine, Furosemide, and dexamethasone [32]. The differences in HNT surface charge have also been exploited for the selective adsorption of anionic and cationic surfactants, which significantly alters HNT zeta potential [33]. Our lab has shown that HNT-coated biomaterials of nanoscale roughness can increase surface area and thus selectin protein adsorption [34], which can enhance selectin-mediated cancer cell capture. Herein, we present the use of HNT in combination with cationic and anionic surfactants to develop nanostructured biomaterials that differentially adhere tumor cells and leukocytes under flow conditions.

2. Materials and methods

2.1. Cell culture

Human colon adenocarcinoma COLO 205 (ATCC #CCL-222), breast adenocarcinoma MCF7 (ATCC #HTB-22), and breast adenocarcinoma MDA-MB-231 (ATCC #HTB-26) cell lines were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). COLO 205 and MDA-MB-231 cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 1% PenStrep (PS), all purchased from Invitrogen (Grand Island, NY, USA). MCF7 cells were cultured in Eagle's Minimum Essential Medium supplemented with 0.01 mg/mL bovine insulin, 10% FBS, and 1% PenStrep, all purchased from Invitrogen. Both cell lines were incubated under humidified conditions at 37 °C and 5% CO_2, and were not allowed to exceed 90% confluence. In preparation for capture assays, cancer cells were removed from culture via treatment with Accutase (Sigma–Aldrich, St. Louis, MO, USA) for 10 min prior to handling. All cells were washed in HBSS, and resuspended at a concentration of 1.0×10^6 cells/mL in HBSS flow buffer supplemented with 0.5% HSA, 2 mM Ca^{2+}, and 10 mM HEPES (Invitrogen), buffered to pH 7.4.

2.2. Polymorphonuclear (PMN) cell isolation

Human neutrophils were isolated as described previously [35,36]. Human peripheral blood was collected from healthy blood donors via venipuncture after informed consent and stored in heparin containing tubes (BD Biosciences, San Jose, CA, USA). Blood was carefully layered over 1-Step™ Polymorphs (Accurate Chemical and Scientific Corporation, Westbury, NY, USA) and separated via centrifugation using a Marathon 8K centrifuge (Fisher Scientific, Pittsburgh, PA, USA) at 1800 rpm for 50 min. Polymorphonuclear (PMN) cells, also known as neutrophils, were extracted and washed in cation-free HBSS, and excess red blood cells were lysed hypotonically. Prior to capture assays, neutrophils were resuspended in HBSS flow buffer supplemented with 0.5% human serum albumin (HSA), 2 mM Ca^{2+}, and 10 mM HEPES (Invitrogen), buffered to pH 7.4.

2.3. Halloysite nanotube functionalization

Halloysite nanotubes (HNT; NaturalNano, Rochester, NY, USA) were added to water to a final concentration of 6.6% (w/v). 1.6 g HNT was added to 100 mL of 0.1 M aqueous sodium dodecanoate (NaL) and 2.4 g HNT were added to 100 mL of 0.1 M aqueous decyl trimethyl ammonium bromide (DTAB; Sigma–Aldrich) and mixed using a magnetic stirrer for 48 h. Surfactant-treated nanotubes were then washed several times in water and allowed to dry overnight. Untreated HNT were kept in water at a concentration 6.6% (w/v). NaL and DTAB-treated HNT were stored in water or methanol respectively, to a final concentration of 6.6% (w/v). To evaluate adsorption of surfactants to HNT, the hydrodynamic radius (nm) and zeta potential (mV) of HNT, NaL-HNT, and DTAB-HNT were measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). Treated and untreated nanotubes at a concentration of 0.37% (w/v) were prepared prior to DLS measurements, using the same solvents as described above for functionalized and untreated HNT samples. To assess the effect of ES adsorption on HNT zeta potential, 0.5 mL of HNT, NaL-HNT, and DTAB-HNT at a concentration of 1.1% (w/v) were centrifuged at 13,000 rpm for 10 min and incubated with 0.5 mL of ES at a concentration of 2.5 μg/mL for 2.5 h at RT. All samples were centrifuged at 13,000 rpm for 10 min and resuspended in water at the same concentration used for HNT measurements in the absence of ES. Colloidal stability of treated and untreated HNT was assessed by allowing samples of treated and untreated HNT to settle for 24 h after mixing.

2.4. Fabrication of nanostructured HNT surfaces

Microrenathane (MRE) tubing (Braintree Scientific, Braintree, MA, USA) of inner diameter 300 μm was cut to 55 cm in length and fastened onto the stage of an Olympus IX-71 inverted microscope (Olympus, Center Valley, PA, USA). Ethanol was rinsed through the tubes using a motorized syringe pump (KDS 230; IITC Life Science, Woodland Hills, CA, USA) at a flow rate of 1 mL/min. To functionalize the inner MRE surface with HNT, microtubes were washed thoroughly with distilled water, followed by incubation with poly-L-lysine solution (0.02% w/v in water) for 5 min and incubation with untreated or NaL-functionalized HNT (NaL-HNT, 1.1% w/v) for 5 min. To functionalize the surface with DTAB-treated HNT (DTAB-HNT), aqueous 2:8 L-glutamic acid (0.1% w/v, Sigma) was incubated in microtubes for 5 min, prior to incubation with DTAB-HNT (1.1% w/v) for 5 min. Microtubes were then washed thoroughly with distilled water at 0.02 mL/min to remove non-adsorbed halloysite, and cured overnight at room temperature (RT). To immobilize ES adhesion protein to HNT-coated surfaces, recombinant human ES/Fc chimera (rhE/Fc) (R&D Systems, Minneapolis, MN, USA) at a concentration of 2.5 μg/mL was perfused through microtubes at 0.02 mL/min. ES was incubated for 2.5 h at RT within HNT-coated microtubes and smooth microtubes in the absence of HNT. In some experiments, HNT-coated surfaces were utilized in the absence of ES. All surfaces were blocked for nonspecific cell adhesion for 1 h via perfusion and incubation with 5% (w/v) bovine serum albumin (BSA, Sigma–Aldrich) at 0.02 mL/min. ES protein was activated with calcium enriched flow buffer for 5 min prior to cell capture experiments.

2.5. ES surface adsorption assay

To characterize ES adsorption on smooth and immobilized HNT surfaces, anti-human CD62E (BD Biosciences, San Jose, California, USA) conjugated to an allophycocyanin (APC) fluorophore was perfused through microtubes at 0.02 mL/min and incubated for 2.5 h at RT, following incubation with ES protein and BSA as described above. Unbound ES antibodies were washed from surfaces using flow buffer. Fluorescent images of adsorbed ES on surfaces were acquired at 90× magnification using an IX-81 inverted microscope linked to a Hitachi CCD camera (Hitachi, Japan). Fluorescent images were analyzed using a three dimensional (3D) surface plot plug-in for ImageJ to obtain pixel intensity data.

2.6. HNT surface characterization

To characterize immobilized HNT surfaces, 100 μL of 1.1% untreated HNT, NaL-HNT, and DTAB-HNT solutions were carefully dried on 3 cm × 3 cm polyurethane (PU) sheets (Thermo Scientific, USA) and sputter coated with Au prior to visualization. SEM images of untreated HNT, NaL-HNT, and DTAB-HNT immobilized onto PU surfaces were acquired with the Leica Stereoscan 440 scanning electron microscope (Leica Microsystems Gmbh, Wetzlar, Germany). For atomic force microscopy (AFM) measurements, flat samples of HNT-coated surfaces were prepared on polystyrene microscope slides (Thermo Fisher Scientific, Rochester, NY, USA) using an 8-well flexiPERM gasket (Sigma–Aldrich) following the same method used for microtube functionalization. Samples were then imaged using a Veeco DI-3000 AFM (Veeco Instruments, Inc., Woodbury, NY). 10 μm × 10 μm images were recorded at random locations on each sample. Three images each of the flat HNT-coated samples and untreated surfaces were analyzed in WSxM 5.0 software [37] to inspect the surface height profiles and root-mean-square surface roughness.