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Carboxybetaine methacrylate oligomer modified nylon for circulating tumor cells capture

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ABSTRACT

Circulating tumor cells (CTC) capture is one of the most effective approaches in diagnosis and treatment of cancers in the field of personalized cancer medicine. In our study, zwitterionic carboxybetaine methacrylate (CBMA) oligomers were grafted onto nylon via atomic transfer random polymerization (ATRP) which would serve as a novel material for the development of convenient CTC capture interventional medical devices. The chemical, physical and biological properties of pristine and modified nylon surfaces were assessed by Fourier transform infrared spectra, atomic force microscope, water contact angle measurements, X-ray photoelectron spectroscopy, protein adsorption, platelet adhesion, and plasma recalcification time (PRT) determinations, etc. The results, including the significant decrease of proteins adsorption and platelets adhesion, as well as prolonged PRTs demonstrated the extraordinary biocompatibility and blood compatibility of the modified surface. Furthermore, we showed that upon immobilization of anti-epithelial cell adhesion molecular (anti-EpCAM) antibody onto the CBMA moiety, the modified nylon surface can selectively capture EpCAM positive tumor cells from blood with high efficiency, indicating the potential of the modified nylon in the manufacture of convenient interventional CTC capture medical devices.

1. Introduction

More than one hundred different cancers arise from almost every tissue or organ in the body. Each year globally, about 14 million people learn they have cancer and 8 million people die from the different cancers [1]. Obviously cancer is one of the most challenging health issues our society are facing nowadays. As our understanding of the complexity and variousness of cancers deepens, the treatment of cancers are evolving from the "one drug fits all" stage to the personalized strategy stage [2]. The personalized treatment requires that a treatment should address a disease not only corresponding to its histology and anatomical site from which it arose, but also according to its specific molecular, genetic or immunologic subtype, thereby lead to the emergence of the so-called personalized cancer medicine [2–6]. As a consequence, various molecular biological and biochemical technologies have been developed and applied in the examination and evaluation of patients trying to answer the question that whether the individual would benefit from a specific therapeutic agents or not [3]. Additionally, the development of new tumor-specific biomarkers that can assist in the selection of targeted therapies as well as assessment of response to therapies are necessary [6]. The biologic characterization of circulating tumor cells (CTC) stands out from all the novel technologies and becomes one of the most important methods due to its ability to provide a profile of an individual patient's tumor, which is required for the guidance of personalized therapy selection and personalized treatment scheme establishment [6].

CTCs, the rare cells with an estimated frequency of one cell for every 10^8–10^{10} peripheral blood cells, are a population of cells found in peripheral circulation that have the potential to act as surrogate source of tumor cells for use in precision medical strategies [3]. By using tumor-specific biomarkers such as anti-epithelial cell adhesion molecular (anti-EpCAM) CTCs in blood from cancers of breast, prostate, pancreas, stomach, and lung can be identified and then prognostic information enabling individualized treatment of cancers can be obtained [7]. Detection of CTCs in peripheral circulation is a minimally or non-invasive method that can help

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elucidate how these cells spread through the bloodstream, settle down at distant sites, and form metastases [8]. The great potential of CTC lies in the promising utility of these rare cells as accessible "fluid biopsy" that would allow frequent, minimally invasive sampling of tumor cells for molecular assays, which are currently being performed with traditional biopsy specimens [9]. For this reason, the capture of CTCs using minimally invasive interventional medical devices from peripheral circulation has become one of the most potential strategies and the modifications by immobilizing biomolecules such as antibodies on current available devices is a promising shortcut [10].

Nylon, commonly known as polyamide in chemistry, has been widely applied in intravascular interventional medical devices [11]. Its outstanding mechanical strength, flexibility, toughness, abrasion resistance as well as high resistance to solvents, oils, and body fluids [12–14] lead to the arousal of interests in its application as CTCs capturing interventional medical devices. However, nylon is less vulnerable to chemical modifications and direct binding of antibodies to the surface due to the relative inert surface while comparing to polymers such as polyvinyl alcohol, cellulose and polysaccharides polymers [15]. "Smart" nylons, which incorporate desirable functionalities, are more and more desired for various applications. The bright side is that several modification methods, such as plasma treatment [16,17], UV irradiation [18,19], N-alkylation [13,20], potassium peroxydisulfate oxidation [21,22], and surface-initiated atom transfer radical polymerization (ATRP) [23,24] are now available for the functionalization of the nylon surfaces. ATRP, as a recently developed controlled "living" radical polymerization method [25,26], has been proved to be a powerful method to prepare well-defined and multiple functional polymer brushes on various surfaces [27–29], including nylon [23,24]. Sequentially, the polymeric brush can provide multiple functional groups for the further antibody bonding, for example, the zwitterionic carboxyl groups.

Zwitterionic polymers containing biomimetic functional groups such as phosphorylcholine, sulfobetaine and carboxybetaine have attracted a lot of attentions for their excellent blood compatibility and antifouling properties [30–33], while carboxybetaine has superior antifouling functionality as compared to sulfobetaine [34,35]. In the previous reports, carboxybetaine polymer brushes have been constructed to create high grafting densities and well-controlled polymer structures on different kinds of surfaces [36,37]. Moreover, the carboxyl groups in carboxybetaine moiety provide sites to immobilize biomolecules, such as proteins and antibodies which is potentially to be applied as CTCs capture devices [10,38].

In this paper, nylon surface was modified by grafting carboxybetaine methacrylate oligomer following ATRP approaches. The physical, chemical and biological properties of pristine and modified surfaces were also studied and reported. Furthermore, by using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide (EDC/NHS) chemistry [38], anti-EpCAM was immobilized on the modified surfaces. The selective capture of gastric cancer cells using the modified nylon were proved. According to our study, the approach of selectively CTCs capture on a nylon surface would be beneficial for the developments of CTCs capture interventional medical devices.

2. Materials and methods

2.1. Materials

2-(N,N′-dimethylamino) ethyl methacrylate (DMAEM, 98%), 3-Chloropropionic acid, copper(I) bromide (CuBr, 99%), 2-bromoisobutyryl bromide (BIBB, 98%), N-hydroxysuccinimide (NHS), N,N,N′,N″,N″-pentamethyldiethylenetriamine (PMDETA, 99%) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Aladdin-Reagent Co. Ltd. (Shanghai, China). Formaldehyde (>36.5%w/w), sodium dodecyl sulfonate (SDS), triethylamine (TEA, 98%), anhydrous toluene were purchased from Sinopharm Chemical Reagent. Rabbit anti human EpCAM monoclonal IgG antibody was purchased from Abcam (ab32392, MA, USA). HRP-conjugated goat anti rabbit IgG and 3,3′-diaminobenzidine chromogen were purchased from Boster Co. Ltd. (Wuhan, China). Hematoxylin, 4′,6-diamidino-2-phenylindole (DAPI), Bovine serum albumin (BSA) and bovine serum fibrinogen (BFG) were supplied by Beyotime Institute of Biotechnology (Nantong, China). Micro BCA Protein Assay Kits were purchased from Thermo Fisher Scientific Inc. (Rockford, USA). Nylon-12 sheets with a thickness of about 1 mm were kindly provided by Freewell Biotechnology Co. Ltd. (Nanjing, China). Platelet-rich plasma (PRP, prepared on a Baxter CS-3000 Plus blood cell separator with 1×10^6 platelets/μL) and platelet-poor plasma (PPP, $<5 \times 10^4$ platelets/μL) of human blood was supplied by Jiangsu Province Blood Center and used within 24 h after collection.

2.2. Instrumentations

Attenuated total reflection Fourier transform infrared spectroscopy (ATR–FTIR) were performed on a Nicolet Nexus 870 FT-IR spectrometer (Thermo Nicolet, USA) from 4000 to 400 cm^{-1} with a resolution of 2 cm^{-1} at room temperature. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker ARX500 NMR spectrometer (Bruker, German) with D_2O as the solvent. X-ray photoelectron spectroscopy (XPS) of samples were recorded on a Thermo Scientific K-Alpha Photoelectron Spectrometer equipped with a monochromatic Al Kα X-ray source to determine the surface elemental composition of each sample; the XPS spectra were acquired in the constant analyzer energy (CAE) mode at a 90° take-off angle. XPS Peak software (v4.1) was utilized to analyze and deconvolute the XPS peaks. Peak deconvolutions were performed using Gaussian components after a Shirley background subtraction. The water contact angles were recorded with a KSV CAM200 optical contact angle and surface tension meter system (KSV Instruments, Finland) at 20 °C with a relative humidity of 60%. Each data point was the average of 10 determinations. A tapping mode NanoScope IIIa-Phase atomic force microscope (AFM) (Digital Instruments, USA) was utilized to observe the micromorphology of the samples.

2.3. Synthesis of CBMA monomer

2-Carboxy-N,N′-dimethyl-N-(2′-methacryloyloxyethyl) ethanaminium inner salt (CBMA) was synthesized by the reaction of DMAEM with sodium 3-Chloropropionate according to literature [39]. NMR was recorded in deuterated water (D_2O) as solvent [40]: ^1H NMR (500 MHz): 6.06 (s, 1H,=CH), 5.68 (s, 1H,=CH), 4.55 (t, 2H, OCH_2), 3.70 (t, 2H, CH_2N), 3.59 (t, 2H, NCH_2), 3.10 (s, 6H, NCH_3), 2.64 (t, 2H, CH_2COO), 1.84 (s, 3H,=CCH_3).

2.4. Surface-initiated ATRP on nylon sheets surfaces

Before the grafting process, nylon sheets ($10 \times 10 \times 1$ mm^3) were boiling for 1 h in 5% NaOH solution and ultrasonically washed by acetone to remove contamination. Then amide groups of the nylon were activated by formaldehyde using a two-step method according to that described before [23]: formaldehyde solution (50 mL) and 85 %wt phosphoric acid (1 mL) were introduced into a 100 mL flask containing 30 nylon sheets. The reaction mixture was kept at 60 °C for 12 h to produce Nylon-OH sheets. After that, the sheets were ultrasonically washed with copious deionized