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Functional dendrimer modified ultra-hydrophilic trapping copolymer network towards highly efficient cell capture

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ABSTRACT

Highly efficient isolation of living tumor cells possesses great significance in research of cancer. Hence, we have designed the 3-aminophenylboronic acid (APBA) derivative dendrimer-functionalized 3D network polyacrylamide/poly (methyl methacrylate) copolymer as capture substrate which is easily prepared, template free and low-cost. The structure of copolymer is compared to "fishing net" in order to increase the contact between cells and substrates. The application of poly (amidoamine) dendrimers provides abundant amino groups to react with APBA which is just like "baits" that can bond with sialic acid in the cytomembrane to realize cell capture. The 3D network structure trammels cancer cells, offers great reaction space and displays hydrophilic surface, which has immensely improved the contact probability of cells and materials. Due to the 3D network structure and dendrimer, this material can achieve a high capture efficiency of 87 ± 5% in 45 min. The viability of captured cells is nearly 100%, as a result of the soft and hydrophilic surface and hypotoxicity of this copolymer.

1. Introduction

Research on the isolation of tumor cells has obtained enormous attention in recent years. As it is reported, different types of tumor cells can be utilized as prognostic markers and its detection can be able to reflect the metastatic relapse and progression in other tumor entities as well [1–6]. Hence, it is pivotal and promising to develop highly efficient and sensitive methods to realize the isolation and detection of tumor cells, which provide a wide platform for the further research on the diagnosis, metastatic mechanisms and therapeutic methods of tumor [7].

In recent years, a diverse range of new techniques have been involved in the task to isolate and detect tumor cells. According to the separation mode, these new methods can be classified as physical isolation and biological affinity isolation [8]. The enrichment of tumor cells by physical properties takes full advantage of the difference in size, deformability and so on [9–11]. However, physical enrichment is short of sensitivity and specificity which limit its application, while biological enrichment often utilizes antibody [12–15], aptamer [16] and other specific markers [17–19] to recognize and capture tumor cells and possesses better efficiency and diversity than physical methods. In terms of biological isolation of tumor cells, microfluidic chips and functional materials such as magnetic beads [12,13], nanostructure array [14–16] and "smart particles" [20,21] are selected as substrates. Research on microfluidic devices has shown that this technique can achieve high capture efficiency by optimization of flow conditions and contact frequency [15,16,22]. Our research group has imitated the principle of microfluidic chip to design the nitrocellulose membrane substrate and also prepared aptamer conjugated magnetic beads [23] for efficient analysis of cancer cells and detecting the captured cells by surface-enhanced Raman scattering imaging [24]. Nevertheless, functional materials obtain unique superiorities in the following respects: high surface area to volume ratio, imitation of biomolecules, porosity [25] and so on, so that it is easier to be modified and contact with cells in rapid response for the functional materials. Especially, three-dimensional network materials display similar structure to the composition of cellular surface, such as microvilli and filopodia [17]. Besides, the polymer provides soft surface that is close to the extracellular matrix [26]. In total, 3D hierarchical polymer is able to offer large space for modification and cell incubation and the network structure can trap cells in the materials to enhance cell contact and capture efficiency of the polymer.

Dendrimers are a series of synthetic macromolecules, which own abundant branches and monodispersity [27]. Nowadays, dendrimers are widely applied in modification of functional materials, for they possess excellent preponderance of biocompatibility, uniformity and a large number of functional ending groups [28]. In this way, functional molecules such as antibodies, aptamer, folic acid and phenylboronic acid can be bonded with dendrimer by conjugation for cell capture. Sialic acid (SA), which has been

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clinically approved as one of the tumor markers, is an anionic monosaccharide that frequently appears at the terminal of glycan chains in eukaryotic cells [29]. Recently researches on sialic acid have manifested that there exists overexpression of SA on membrane glycoproteins and glycolipids in tumor cells of various origins [30]. As a result, overexpression of SA on the membrane has been selected as an implication of the malignant and metastatic phenotypes for several different types of cancers [29]. Phenylboronic is widely utilized as the select targets of SA [31], for it can form a stable complex with sialic acids [18,19,32], benefitting from the diols-containing targets existing in the sialic acids [33].

As it is reported, the polyacrylamide possesses network structure [34], biocompatibility [35], hydrophilicity [36] and this material is easy to obtain. The polyacrylamide has been widely used in gel electrophoresis, drug-release, oilfield chemical assistants and so on [37,38]. However, this polymer has been rarely applied in the isolation of tumor cells. Benefited from the characteristics of polyacrylamide, we designed functional polyacrylamide (PAM)/poly (methyl methacrylate) (PMMA) copolymer as substrates, followed by the modification of dendrimers and 3-aminophenylboronic acid (APBA) in sequence. Through optimization of experimental conditions, the functional dendrimer modified 3D network copolymer could achieve a high capture efficiency and viability due to the soft and hydrophilic surface as well as the particular network structure which supported our idea and provided a good method to capture cells.

2. Experimental section

2.1. Materials

Acrylamide, ammonium persulfate (APS), Methyl methacrylate (MMA) and polyethylene glycol (PEG-6000) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Tetramethylethylenediamine (TEMED) was acquired from Beijing Biodee Biotechnology Co., Ltd. Bis-Acrylamide was bought from Sino-American Biotechnology Co. The chemical reagents above were utilized to synthesize the copolymer. Dendrimer (PAMAM 5.0), 3-aminophenylboronic acid (APBA), N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride Crystalline (EDC), N-Hydroxysuccinimide (NHS), glutaraldehyde and sodium cyanoborohydride, which were applied in modification of copolymer, were obtained through Sigma-Aldrich Co., LLC. Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), trypsin and phosphate buffer saline (PBS) were purchased from Shanghai solarbio Bioscience & Technology Co., LTD. Fluorescent dyes involved in our experiments were as follows: 4′,6-diamidino-2-phenylindole (DAPI), acridine orange (AO), prodium iodide (PI), and alizarin red S (ARS), they were provided by Sigma-Aldrich Co., LLC. U251 cancer cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). SEM images were taken by Philips XL30. FT-IR spectrum data was obtained from Nicolet Nexus 470. UV absorption spectra was measured with UV spectrophotometer (Agilent 8453). Fluorescence images of cells and materials were photographed by Leica DM4000 B LED.

2.2. Polymerization of polyacrylamide/poly (methyl methacrylate) copolymer

Firstly, we chose 96-well plate as reactors to shape the copolymer. Then we prepared mixed solution as follows: 20 mg acrylamide, 20 mg bis-acrylamide and 30 mg PEG-6000 were dissolved in 600 μL PBS solution (pH 7.4) under ultrasonic treatment. Each well was added in 50 μL of the solution above, 1.068 μL methyl methacrylate, 0.6 μL TEMED and 2.5 μL APS solution (0.1 g/mL). After this procedure, the 96-well plate was placed in a constant temperature vibrator (50 °C, 100 rpm) for 1 h. The products were washed with PBS for three times. The physical state of the copolymer changed from liquid to solid and the shape of this white PAM-PMMA copolymer was just fitted with the 96-well plate.

2.3. Synthesis of dendrimer-functionalized copolymer

Before modification, the copolymer was exposed under UV for 10 min and washed with PBS. The copolymer was activated by EDC solution (14 mg/mL in PBS, pH 7.4) for half an hour, then added 7.5 μL PAMAM in it. During 2 h of reaction, the dendrimer-functionalized copolymer was generated and should be washed with PBS for three times.

2.4. Preparation of APBA modified dendrimer-copolymer

Briefly, the dendrimer-copolymer was activated in glutaraldehyde solution (5%) for 2 h at first. Subsequently, the activated copolymer was added in APBA (10 mg/mL) and reacted for 3 h. After that, the copolymer was washed with PBS. Finally, the product was immersed in PBS which contained $NaBH_3CN$ (10 mg/mL) for 1 h to end the reaction. The APBA modified dendrimer-copolymer was synthesized and took on a claybank surface while the surface of non-modified material was white.

2.5. Capture assay towards cancer cells

To characterize the great potential of the materials in cell capture, we conducted several groups of parallel capture experiments to optimize the properties of materials and the capture conditions. In this task, U251 cell line was selected as targeted tumor cells for the reason that sialic acids overexpressed in its cytomembrane. The cells were incubated at 37 °C, 5% CO_2 in DMEM which was supplemented with 10% FBS. As the information mentioned above, the APBA modified dendrimer-copolymer was synthesized in 96-well plate which was convenient for cell capture and image, so we injected 100 μL cell suspension at a density of 10^5 cells per mL in the 96-well plate and incubated at 37 °C, 5% CO_2 for 45 min. After incubation, the supernate was sucked up and the materials bonded with U251 were rinsed by PBS (pH 7.4) for three times, all the washing solution were reserved for the calculation of cell capture efficiency.

2.6. Test of the viability of captured cells

AO and PI [26] were used to evaluate the viability of captured U251 cells. The stock solution of AO/PI was prepared by using PBS (pH 7.4, without calcium and magnesium) to dilute the dye liquor from 1 mg/ml to 10 μg/mL, respectively. After incubation and rinse, the materials were added in equivalent amount of AO and PI stock solution and reacted for 15 min. The dyed cells were rinsed by PBS for several times, then observed by fluorescence microscope.

2.7. Characterization

To describe the morphology of copolymer, we used scanning electron microscope (SEM). The components of copolymer were preliminarily examined by Fourier transform infrared spectroscopy (FTIR). The elemental analysis was conducted through energy dispersive spectrometer (EDS) to evaluate the APBA modified dendrimer-materials.

To testify whether the dendrimer was successfully modified onto the copolymer, we applied ultraviolet absorption spectrum to