Selective Cancer Cell Isolation from Blood using Thermoresponsive Imprinted Hydrogel with Switchable Sialic Acid Recognition

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Abstract

In this study, a thermoresponsive hydrogel layer imprinted with Sialic Acid (SA) was created for the purpose of selectively capturing and releasing cancer cells. Using a thermoresponsive functional monomer, the SA-imprinting procedure was carried out at 37°C, resulting in switchable SA-recognition sites with potent SA binding at 37°C and poor binding at a lower temperature (for example, 25°C). The SA-imprinted hydrogel layer may be exploited for the selective recognition of cancer cells since SA is frequently overexpressed at the glycan terminals of cell membrane proteins or lipids. Our findings demonstrated that the hydrogel layer was effective at removing cancer cells from both real blood samples and culture medium. Also, by lowering the temperature, the collected cells could be released without any harm. This thermo-responsive hydrogel layer could be used as a promising and versatile platform for cell-based cancer diagnosis due to its non-invasive processing mode, high capture efficiency, good cell selectivity, and more stable and durable SA-imprinted sites compared to natural antibodies or receptors.

Keywords: Molecular imprinting • Cell capture • Thermo-responsive hydrogels • Molecular recognition • Sialic acid

Introduction

A number of fundamental events that are essential to several biological processes at the molecular and cellular levels are represented by molecular recognition in living systems. Reversible alterations in these natural molecular recognition systems, such as the receptor-ligand or antibody-antigen interactions, will take place as a result of external or internal biological stimuli. These dynamic interactions then set off particular intracellular and cell-signaling cascades, which ultimately lead to normal or pathological cellular activities. In other words, both physiology and pathology in biosystems depend on the molecular recognition-mediated cellular processes. In this context, the creation of sophisticated biomaterials and biomedical devices has drawn growing attention in molecular recognition. Selective incorporation of natural molecular recognition into non-biogenic materials has been shown to be a successful method for enhancing their initiative to communicate with cells in biomaterial design and bioengineering. The increased vitality could greatly promote the biological performance of materials or devices, such as improving biocompatibility or accelerating tissue regeneration. These natural molecular recognition systems (such as receptor-ligand or antibody-antigen interactions), despite significant advancements in biomaterial production, nevertheless have intrinsic limitations in real-world applications. Its separation and purification are frequently time-consuming and expensive, in addition to their physicochemical instability and short shelf life. Because of these disadvantages, researchers are searching for more reliable replacements with molecular specificity resembling receptors or antibodies. In order to duplicate the molecular specificity of receptors or antibodies, a number of synthetic approaches have been developed recently. Since the resulting Molecularly Imprinted Polymers (MIPs), which resemble natural receptors and antibodies and are molecularly programmable, reversible, and selective, a great deal of criticism. The idea of molecular imprinting was founded on how antibodies are made and how enzymes catalyse reactions. Molecular templates with functional monomers and crosslinkers self-assemble and imprint, resulting in the creation of molecular recognition sites in MIPs. These imprinted sites are spatially complementary to the form and function of the template molecules, much like natural molecular recognition systems. MIPs have applications in a variety of fields, including as separation, immunoassays, catalysis, drug delivery, sensing, bio-imaging, and so on, because to their high physicochemical stability, adaptable molecular specificity, low cost, and ease of production. MIPs were created with the purpose of simulating spontaneous molecular recognition that frequently takes place in cellular processes, as is well known. Yet, the applications of modern MIPs at the cellular level are still novel. A notable difference is that while cell separation based on the molecular specificity of MIPs has rarely been investigated, molecular separation and extraction utilising MIPs have experienced tremendous success. In actuality, cell divisions play a crucial role in both basic cell biology and contemporary medical diagnostics. For instance, cancer theranostics has showed considerable potential with the selective extraction of cancer cells from blood stream (i.e., Circulating Tumour Cells, CTCs). This is due to the fact that the study of CTCs will yield crucial data for the early diagnosis and prognosis of cancer. As a result, the creation of a MIPs-based platform for selective cancer cell isolation is crucial for both the advancement of medical theranostics as well as the functioning replications of natural molecular recognition in biosystems. Imprinting a particular biomarker on the outer layer of cell membrane is one of the rational ways to generate MIPs with cell recognition. Sialic Acid (SA), which has been revealed to be a significant component of the glycans of cell membrane proteins or lipids, has been linked to a number of disease conditions, most notably malignancies. The ability to create cancer affinity by designing MIPs with specific SA-recognition is thus provided by the overexpression of SA residues on cancer cell surfaces. This process has been employed for targeted cancer cell imaging employing the SA-imprinted nanoparticles. These examples demonstrated the significance of the SA-imprinted sites in MIPs for the detection of particular cancer cells. Theoretically, these sites may also function as a selective factor for cancer cell separation. In light of this, we continue with our research and aim to use the particular SA-recognition of MIPs for picky cancer cell separation. This research will expand the use of artificial molecular recognition (i.e., MIPs) from straightforward molecular separation or extraction to selective cellular isolation, which holds promise for the early diagnosis and prognosis of cancer based on cell-based tumours. In our design, we'll make a layer of hydrogel that has been imprinted with SA and look into how the identification of SA causes cancer cells to adhere to the surface. Thermoresponsiveness is involved in this system to produce switchable SA-recognition through a temperature transition approach, which will aid in future cell detachment and harvesting. Surface SA-recognition will cause selective cell capture when cancer cells are treated with the thermoresponsive hydrogel layer at 37 °C. When the temperature is lowered to 25°C, the deteriorating SA-recognition brought on by the altered shape and functionality of the SA-imprinted spots will cause the attached cancer cells to escape quickly. MIPs-based molecular recognition is less expensive and more robust than natural analogues, and in this study, the molecular selectivity towards SA could be modified via thermo-switchability. As a result, we think the study could serve as a model for creating cell isolation platforms using chemically tailored molecular recognition. Also, the low- or non-invasive cell harvesting is

made possible by the physiologically appropriate thermo-responsiveness, demonstrating the superiority of this study's ability to accurately diagnose cancer, which is greatly dependent on the condition of the cancer cells that were harvested. HepG-2 human liver cancer cells (obtained from Shanghai Cell Center, with overexpressed SA on the cell surface) were grown in RPMI 1640 media with 10% foetal bovine serum and 1% penicillin-streptomycin. Fibroblasts (L929 cells) were grown in MEM medium with 10% foetal bovine serum and 1% penicillin-streptomycin (obtained from Shanghai Cell Center). Every cell was cultivated at 37 °C in a 5% CO2 environment. After achieving sub-confluency, the medium was changed three times each week, and the cells were collected using 0.25% trypsin and 0.26 mM EDTA in PBS. HepG-2 cells were pre-stained with DiO (green) and L929 cells with DiI (red) prior to cell collection. A 24-well plate containing the hydrogel layers SIH and NIH was filled with 1 mL PBS for storage. After removing the PBS, add 1 mL of HepG-2 cells (5 104 cells/mL) that have been pre-stained with DiO to each well. Incubate each mixture at 37°C for 1 hours, 1.5 hours, 2 hours, and 2.5 hours, respectively. The medium was then taken out, and the minimally adsorbed cells were gently rinsed with PBS. The collected cells were then counted on the SIH and NIH, respectively. The trapped cells were then cooled at 25 °C for 30 min for the cell release experiment, mildly washed with PBS, examined under a fluorescence microscope, and counted. The captured cells were measured using a digital camera and scaled up to get the total number of captured cells. Then, a live/dead experiment was performed on the original and recovered cells by staining the living and dead cells with AO (green, 20 g/mL), and PI (red, 15 g/mL), respectively. With a fluorescence microscope, the stained cells were analysed. In a 6-well plate, 1,104 original cells and recovered cells were subcultured. The condition of cell adhesion was examined under a microscope following 1 day, 3 days, or 5 days of incubation in the medium at 37°C, and cell proliferation was assessed using the CCK-8 assay. Each well received 100 L of CCK-8 solution, and cells were then cultured at 37°C for 24 hours. In order to determine the absorbance, a microplate spectrophotometer was used at 450nm. Six parallel tests were conducted.

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