DEVELOPMENT OF NOVEL CELL SEPARATION SYSTEM USING POLY(*N*-ISOPROPYLACRYLAMIDE)-*GRAFT*-POLYPROPYLENE NON-WOVEN MEMBRANE WITH ANTIBODY

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Introduction

Many types of cell-separation methods have been studied and developed[1]. However, these methods are expensive, cannot be operated continuously on a large scale, and are troublesome. The purpose of our study is to develop a novel cell separation method that can capture or enrich a specific cell type continuously and in high yield and that can be operated easily and quickly in clinical settings.

We have designed a novel cell-separation system that uses a poly(*N*-isopropylacrylamide)-*graft*-polypropylene (PNIPAAm-*g*-PP) membrane containing an adsorbed monoclonal antibody that binds specifically to the surface of the target cells. Non-woven poly(propylene) (PP) membrane has many advantages, such as large specific surface area, chemical and physical stability, good resistance to biodegradation, and low cost. Poly(*N*-isopropylacrylamide) (PNIPAAm) has its unique thermal properties: PNIPAAm undergoes a reversible thermoresponsive phase transition in an aqueous solution at approximately 32°C (the lower critical solution temperature, LCST). PNIPAAm is insoluble (in a hydrophobic state) in water above the LCST and reversibly soluble (in a hydrophilic state) below the LCST.

We previously prepared a PNIPAAm-*g*-PP membrane by plasma-induced graft polymerization and found that the permeability of water through this membrane could be controlled by changing the temperature around 32°C[2]. We hypothesized that a PNIPAAm-*g*-PP membrane adsorbed monoclonal antibody (mAb) would selectively capture a specific cell type having a certain receptor on its surface above the LCST and released the captured cells below the LCST as shown schematically in Figure 1.

We previously reported that the PNIPAAm-*g*-PP membrane containing antibody had the separation ability from a mixture of cells, which had adherent property[3]. In this study, we investigated the ability of these PNIPAAm-*g*-PP membranes to selectively separate a specific cell type from a mixture of non-adherent cell types. In addition, the mechanism of separating cells was elucidated.

Selective adhesion of CD34-positive, human bone marrow acute myelogenous leukemia cells (KG-1a) to PNIPAAm-*g*-PP membranes containing adsorbed anti-human CD34 mAb was examined. Next, the detachment of attaching cells from the PNIPAAm-g-PP membrane by changing the temperature was evaluated. The ability of the PNIPAAm-*g*-PP membranes containing anti-human CD34 mAb to selectively separate cells from a 1:1 suspension of KG-1a cells and CD-34 negative, human T lymphoma cells (Jurkat) was confirmed. Finally, the mechanism of this cell separation was discussed focusing on the functions of the mouse IgG1, which was used as a model of mAb, and the grafted PNIPAAm chains.

Experimental

N-isopropylacrylamide (NIPAAm) was dissolved in water (3 wt%) and used as the monomer solution. Graft polymerization of NIPAAm on the PP membrane was carried out as before[2,3].

PNIPAAm-*g*-PP membrane disks were treated with or without anti-human CD34 mAb in PBS (0, 5, 10, or 20 μg/mL). KG-1a cells or Jurkat cells were incubated on the membranes for 45 min at 37°C in RPMI1640 medium without serum. After washing with PBS at 37°C, the attaching cells were fixed in methanol, stained with Giemsa, and counted to evaluate cell adhesion to the membrane.

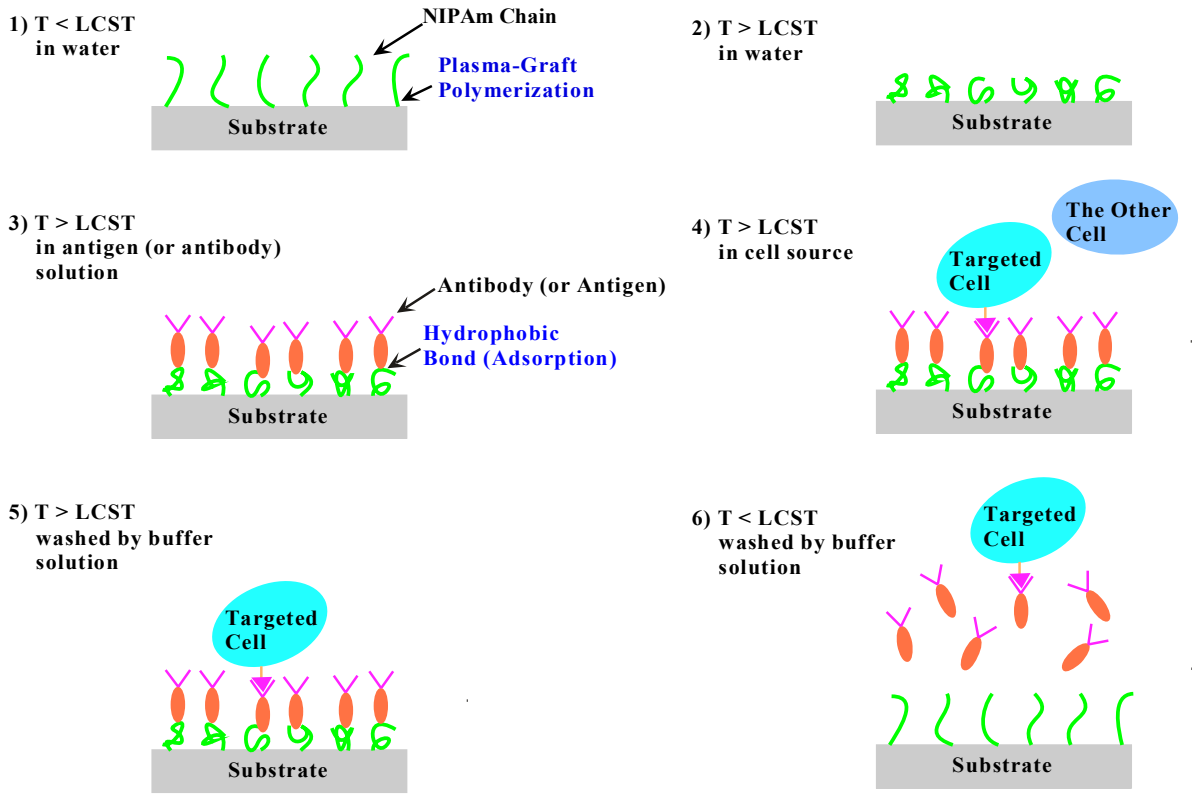

Figure 1. Schematic mechanism of cell separation using PNIPAAm-*g*-PP membrane.

A 1:1 suspension of KG-1a cells and Jurkat cells in RPMI1640 medium without serum was incubated on anti-human CD34 mAb-treated PNIPAAm-*g*-PP or PP membrane disks, for 45 min at 37°C. The membrane disks were washed with PBS at 37 °C, and then washed at 10°C for 15min in the medium. Before or after washing, the number of cells attaching to the PNIPAAm-*g*-PP or PP membranes was counted. The viability of detached cells was determined by trypan blue exclusion assay. The detached cells were stained with FITC-conjugated anti-human CD34 mAb and were counted with a confocal laser microscope. Cell separation efficiency was calculated as the relative yield of KG-1a cells.

Diluted mouse IgG1 (10 μg/mL, PBS) was added to a PNIPAAm-*g*-PP membrane disk at 25°C, and then was incubated in for 1.5 h at 37°C with shaking. The disk was washed with PBS 4 times at 37°C. The disk was washed in 0.1% BSA (PBS) with shaking at 37°C or 10°C for 15 min at the same time. Detection of the remained mouse IgG1 the membrane surface was carried out using enzyme-linked immunosorbent assay (ELISA) method.

Results and Discussion

As the concentration of CD34 mAb increased, cell adhesion of KG-1a cells to PNIPAAm-*g*-PP membrane containing adsorbed CD34 mAb increased (Figure 2). Cell adhesion of KG-1a cells to PNIPAAm-*g*-PP membrane containing adsorbed anti-human CD34 mAb was much higher than that of Jurkat cells to PNIPAAm-*g*-PP membranes (Figure 3). Figure 4 shows a scanning electron micrograph of KG-1a cells attaching to a PNIPAAm-*g*-PP membrane containing adsorbed anti-human CD34 mAb. The cells appear to bind the fiber surface at multiple points.

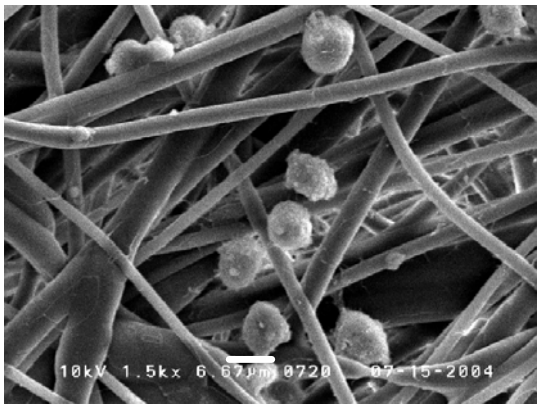

Figure 4. Scanning electron micrograph of KG-1a cells adhering to PNIPAAm-*g*-PP membrane that had been soaked in anti-human CD34 mAb (10 μg/mL PBS) at 37 °C for 2 h. Scale bar = 6.67 μm.

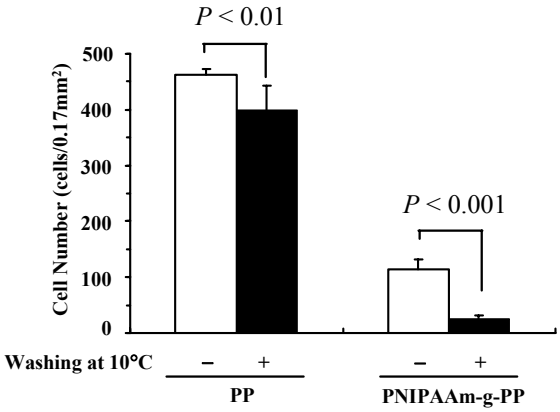

Figure 5. Effect of washing at 10°C on detachment of adhering cells from the PNIPAAm-*g*-PP membrane or PP membrane containing adsorbed CD34 mAb.

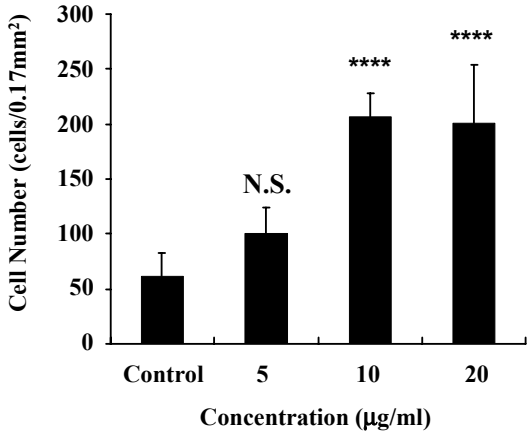

Figure 2. Effect of concentration of anti-human CD34 mAb added to PNIPAAm-*g*-PP membranes on later adhesion of KG-1a cells to these membranes (n = 4). ****P < 0.0001.

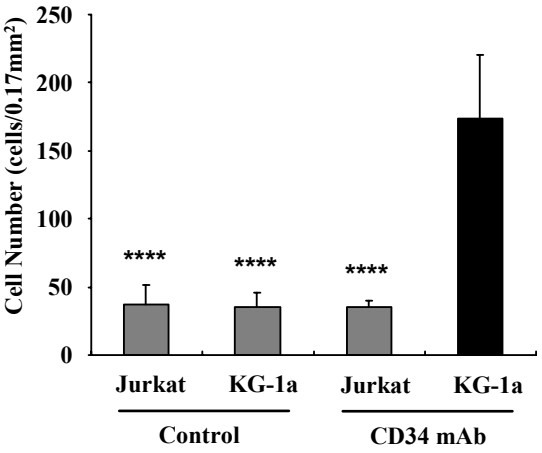

Figure 3. Adhesion of KG-1a cells or Jurkat cells to PNIPAAm-*g*-PP membranes containing adsorbed anti-human CD34 mAb (n = 4). ****p < 0.0001.

After washing at 10°C, only 13% of the cells attaching to PP membranes containing adsorbed anti-human CD34 mAb were detached (Figure 5). In contrast, 77% of the cells attaching to PNIPAAm-*g*-PP membranes containing adsorbed anti-human CD34 mAb were detached by washing at 10°C.

Laser scanning confocal observations of fluorescence emitted from cells recovered from PNIPAAm-*g*-PP membranes containing adsorbed anti-human CD34 mAb showed that more cells emitted FITC-derived fluorescence (from anti-CD34 mAb) than cells emitting no fluorescence. Consequently, the CD34 positive, KG-1a cells were enriched from 50% in the original cell suspension to 76% of the recovered cells by using the membrane with the CD34 mAb (Figure 6). The viability in the recovered cells was 91%.

Figure 7 shows that the amount of mouse IgG_1 remained to the membrane after washing at 10°C was almost the same amount of mouse IgG_1 remained to the membrane after washing at 37°C. This result shows that desorption of mouse IgG_1 was hardly promoted by washing at 10°C, below LCST, compared to by washing at 37°C above LCST.

Cell adhesion of KG-1a cells to PNIPAAm-*g*-PP membrane containing adsorbed anti-human CD34 mAb was significantly promoted. This finding suggests that merely soaking a PNIPAAm-*g*-PP membrane in a solution containing the antibody is sufficient to specifically capture the required cell type.

Reducing temperature below the LCST causes the grafted PNIPAAm chain to be hydrated and the surface of the membrane to become hydrophilic, which should favor detachment of the attaching cells.

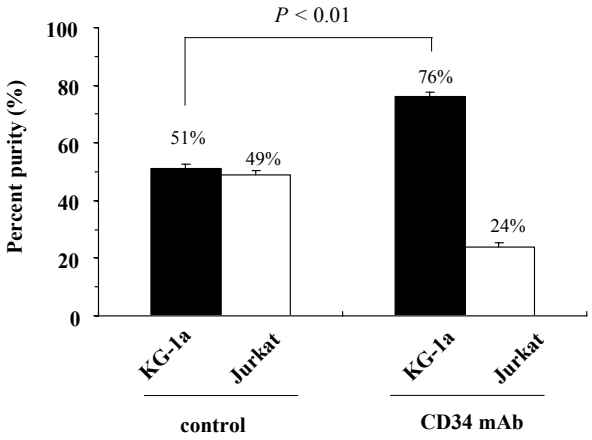

Figure 6. Percent purity of KG-1a cells and Jurkat cells in cells recovered from a 1:1 mixed cell suspension by separation with PNIPAAm-*g*-PP membranes containing adsorbed anti-human CD34 mAb (n = 4).

With this novel method, we succeeded in enriching non-adherent cell type (KG-1a cells) non-invasively. The IgG_1 adsorbed to the PNIPAAm-*g*-PP membrane was not enhanced to detach by reducing temperature, although the cells attaching to the PNIPAAm-*g*-PP membrane was enhanced to detach. From these results, mAb was considered to adsorb to the un-graft region on PNIPAAm-*g*-PP membrane and to cause the adhesion of specific cell types. Hydration of PNIPAAm-*g*-PP chain was considered to cause cell detachment from the membrane.

Conclusions

The results obtained in the present study indicate that a PNIPAAm-*g*-PP membrane containing adsorbed antibody has the potential to separate or enrich target cells from a non-adherent cell suspension non-invasively with ease. Selective cell adhesion on the PNIPAAm-*g*-PP membrane depends on using an adsorbed antibody specific to the desired target cell. Detachment of attaching cells from the membrane is facilitated by the thermosensitive poly(NIPAAm) chains on the PP non-woven membrane.

References

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3. Okamura, A., Itayagoshi, M., Hagiwara, T., Yamaguchi, M., Kanamori, T., Shinbo, T., Wang, P.-C., "Poly(*N*-isopropylacrylamide)-*graft*-polypropylene membrane containing adsorbed antibody for cell separation, *Biomaterials*, **26**, 1287-1292, 2004.