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# Thermally responsive microcarriers with optimal poly(*N*-isopropylacrylamide) grafted density for facilitating cell adhesion/detachment in suspension culture

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ABSTRACT

Large-scale cell culture of anchorage-dependent cells based on microcarriers is a crucial method for industrial-scale cell culture and large-scale expansion of therapeutic cells. Previously, the authors developed temperature-responsive microcarriers bearing poly(*N*-isopropylacrylamide) (PIPAAm)-grafted chains on their outer surface for the non-invasive detachment of cultured cells through temperature reduction without proteolytic enzyme treatment. In this study, to further facilitate cell adhesion and thermally induced detachment efficiency, PIPAAm-grafted beads with various grafted amounts and various grafted PIPAAm chain densities were prepared. Contact angle measurements at different temperatures revealed that the magnitude of the contact angle change from 37 to 20 °C decreased with increasing brush density. Additionally, the amount of fibronectin adsorbed on the bead surface decreased with increasing brush density. Chinese hamster ovary (CHO-K1) cells adhered to the surface of PIPAAm-grafted beads at 37 °C, and a negligible difference in the cell adhesive property was observed by varying the brush density of the PIPAAm-grafted beads. When the temperature was reduced to 20 °C, the adhering cells were found to detach themselves from the PIPAAm-grafted bead surfaces. Of particular interest, PIPAAm-grafted beads with intermediate brush density exhibited the highest efficiency of thermally induced cell detachment. Thus, the brush density of PIPAAm-grafted beads strongly affected the efficiency of thermally induced cell detachment.



## 1. Introduction

Microcarrier culture (cell cultivation on the surface of microbeads, often referred to as microcarriers) in stirred suspension, is currently regarded as an essential large-scale suspension culture method that can be used with a broad range of anchorage-dependent cells [1–3]. Owing to the large surface area-to-volume ratio involved, microcarrier surfaces provide a large area for monolayer cultured cells and provide the maximum achievable cell density in a space-saving and cost-effective manner compared with two-dimensional planar surfaces. This method has been employed for the industrial-scale production of pharmaceutical recombinant proteins, such as antibodies [1,2]. Moreover, microcarrier culture is also regarded as a potential large-scale culture method for obtaining a sufficient number of therapeutic cells for regenerative medicine [3–5], as large numbers of cells are needed to complement whole human tissues. In this regard, the scalable culture of therapeutically useful stem cells, including bone marrow-derived mesenchymal stem cells (MSC) [6,7], and cells differentiated from embryonic stem cells [8] has been demonstrated based on microcarrier culture. In these studies, various commercially available microcarriers consisting of dextran, glass and poly(styrene) with a diameter range of ~100–200 μm were used [3–5]. However, conventional microcarriers are designed to maximize cell adhesion and proliferation properties. Therefore, repeated trypsinization is required during passaging culture to harvest the adhering cells from the surface of the microcarriers. This proteolytic enzyme treatment degrades plasma membrane proteins and the extracellular matrix (ECM) [9,10], leading to a reduction in cell viability and/or reattachment efficiency. Additionally, in view of the application of microcarrier culture to the large-scale culture of therapeutic cells, the use of animal-derived enzymes should be avoided to prevent possible contamination with adventitious agents. Thus, a non-invasive cell harvest method that does not require the use of proteolytic enzymes would be an advantageous and promising method for large-scale microcarrier culture of therapeutic cells.

In this regard, the present authors' laboratory has developed temperature-responsive microcarriers bearing poly(*N*-isopropylacrylamide) (PIPAAm)-grafted chains on their outer surface for harvesting cultured cells using temperature alteration rather than proteolytic enzyme treatment (Fig. 1A) [11]. Cells can adhere to and proliferate on the surface of PIPAAm-grafted beads at 37 °C, a temperature that is greater than the lower critical solution

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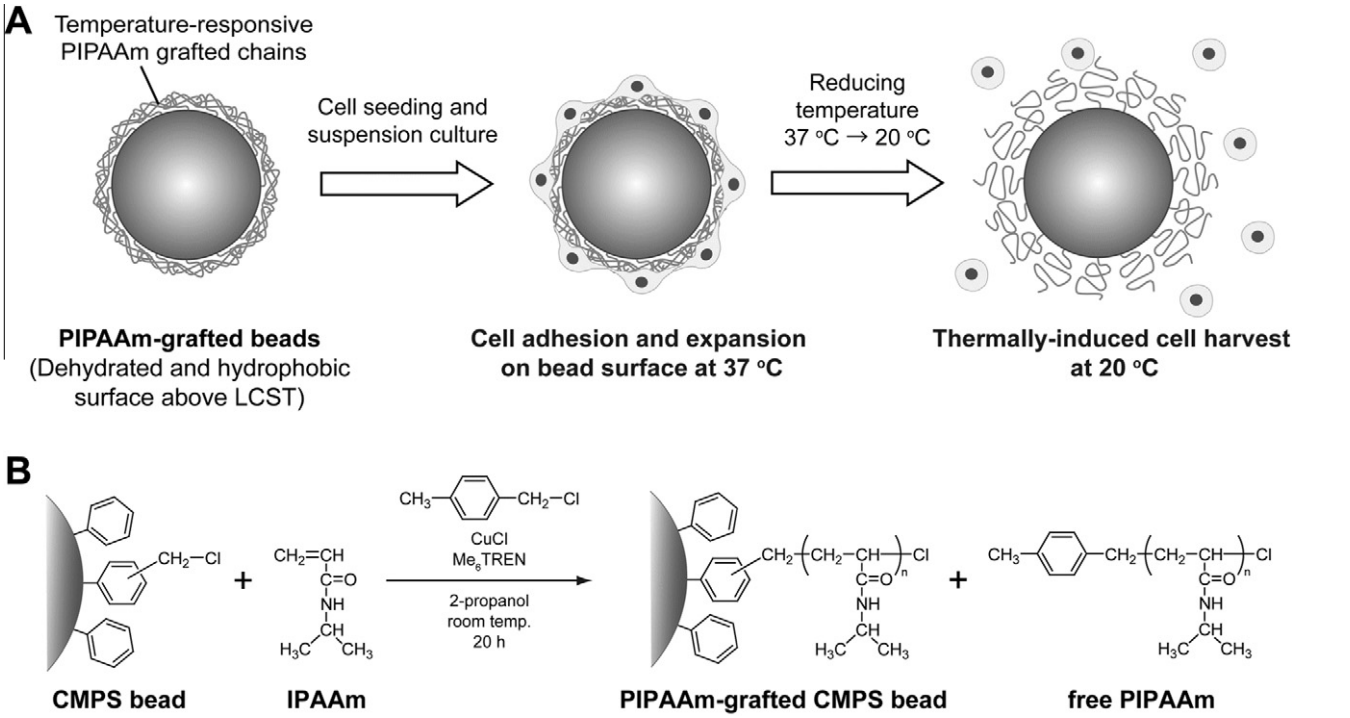


**Fig. 1.** (A) Schematic illustration of PIPAAm-grafted microcarriers capable of temperature-dependent cell adhesion and detachment for large-scale suspension culture. (B) The preparation scheme of PIPAAm-grafted CMPS beads by SI-ATRP in the presence of soluble free initiator.

temperature (LCST) of the PIPAAm homopolymer (~32 °C). In sharp contrast, by reducing the temperature below the LCST (20 °C) and allowing the surface to become hydrophilic, the adhering cells spontaneously detach themselves from the surface without the need for proteolytic enzyme treatment. Previous research reveals that the amount of PIPAAm grafted and the diameter of the beads strongly affect cell adhesion, proliferation and thermally induced detachment behavior [11]. Efficient cell proliferation in stirred suspension culture and subsequent thermally induced non-invasive harvest are achieved by the use of optimized 100-μm-diameter microcarriers with adequate amounts of PIPAAm grafted onto them. Herein, this PIPAAm-grafted microcarrier is prepared by a surface-initiated atom transfer radical polymerization (SI-ATRP) method, which yields a densely grafted brush layer on the surface [12]. Detailed atomic force microscopy and neutron reflection studies reveal that the magnitude of the temperature-dependent conformational change and the hydration/dehydration change across LCST are strongly influenced by the grafted density of PIPAAm chains [13–15]. Because the temperature-dependent conformational change and the hydration/dehydration change in the grafted PIPAAm chains play a pivotal role in regulating the interaction with proteins and cells, the effect of the PIPAAm grafting density needs further investigation to improve the temperature-responsive microcarrier system. In fact, few cells adhere on densely grafted long PIPAAm brushes on flat substrates at 37 °C, mainly owing to their effect on repelling the adsorption of ECM components, such as fibronectin, on the partially hydrated and collapsed PIPAAm brush surfaces [16–18]. However, the effect of the grafting density of PIPAAm when used in PIPAAm-grafted microcarriers on cell adhesion and thermally induced detachment has not yet been studied [11].

In this study, to further optimize cell adhesion and detachment control, the effects of the grafting density of PIPAAm on cell adhesion and thermally induced detachment were investigated. Temperature-responsive PIPAAm-grafted microcarriers with various grafted amounts and densities of PIPAAm chains were synthesized by SI-ATRP using chloromethylated poly(styrene) (CMPS) beads with varying chlorine contents (Fig. 1B). Basic measurements, including surface property and cell attachment/detachment studies of the beads, revealed that the PIPAAm grafting density significantly affected the thermally induced cell detachment behavior. Optimization of the grafting density of PIPAAm on bead surface would contribute to large-scale cell culture and yield important insights into the practical use of other temperature-responsive smart biomaterials.

## 2. Materials and methods

### 2.1. Materials

CMPS beads with mesh size 100–200 mesh and with varying chlorine content were obtained from Aldrich (Milwaukee, WI, USA) (chlorine content 3.8 mmol $g^{-1}$) and Tokyo Chemical Industry (Tokyo, Japan) (chlorine content 2.4 mmol $g^{-1}$ and 1.2 mmol $g^{-1}$). The average diameter, coefficient of variation, specific surface area and chlorine content of the beads are summarized in Table 1. *N*-

**Table 1**
Characterization of CMPS beads with various chlorine content used in this study.

| Code | Bead diameter (μm)[a] | Coefficient of variation (%)[b] | Specific surface area ($cm^2\ g^{-1}$)[c] | Chlorine content[d] | |
|---|---|---|---|---|---|
| | | | | (mmol $g^{-1}$) | (wt.%) |
| CMPS-1.2 | 100.0 ± 16.9 | 16.7 | 643 | 1.2 | 4.3 |
| CMPS-2.4 | 98.6 ± 16.3 | 16.5 | 603 | 2.4 | 8.5 |
| CMPS-3.8 | 108.9 ± 16.5 | 15.2 | 647 | 3.8 | 13.4 |

[a] Abbreviated as CMPS-*X*, where *X* represents the chlorine content of the beads (mmol $g^{-1}$).
[b] Expressed as the mean ± SD; $n = 289$ for CMPS-1.2, $n = 256$ for CMPS-2.4, and $n = 280$ for CMPS-3.8.
[c] Determined using the Brunauer–Emmett–Teller (BET) Kr gas adsorption isotherm.
[d] Determined from halogen elemental analysis.

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