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# Temperature-responsive poly(*N*-isopropylacrylamide)-grafted microcarriers for large-scale non-invasive harvest of anchorage-dependent cells

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ABSTRACT

Cell cultivation on the surface of microcarriers in stirred suspension is an essential method for the large-scale culture of anchorage-dependent cells. For applying this method to the field of cell therapy and for obtaining a large number of intact cells, non-invasive cell harvest without proteolytic enzyme treatment is an advantageous method. In this regard, temperature-responsive microcarriers that bearing poly(*N*-isopropylacrylamide) (PIPAAm)-grafted chains on the outermost surface were developed for harvesting cultured cells by temperature alteration. PIPAAm-grafted beads with the various grafted amount of PIPAAm and various bead diameters were synthesized for optimizing cell proliferation and thermally-induced detachment on the surface. The chinese hamster ovary (CHO-K1) cells adhered on the surface of all PIPAAm-grafted beads at 37 °C, while the adhering cells were found to detach themselves from the surfaces at 20 °C. The efficiency of thermally-induced cell detachment increased with increasing the grafted amount of PIPAAm and the diameter of bead. An efficient cell proliferation on bead surfaces in stirred suspension culture and subsequent thermally-induced cell detachment were achieved by the precise regulation of both the grafted amount of PIPAAm and diameter of bead. The temperature-responsive microcarriers exhibiting temperature-dependent cell adhesion and detachment will be an attractive candidate for the large-scale cell culture of therapeutic cells.



## 1. Introduction

Regenerative medicines including tissue engineering and cell transplantation are promising cytotherapeutic approaches for regenerating damaged or defected tissues as well as treating various intractable diseases [1–3]. To achieve a successful regeneration of the broad range of human tissues, numerous number of cells ranged from $10^9$ to $10^{11}$ are required to replace large human tissues, meaning that the establishment of large-scale cultivation methods for expanding therapeutic cells including pluripotent stem cells and somatic cells to therapeutically useful number is an essential issue [4,5]. For the large-scale cultivation of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), a suspension culture system is demonstrated for the formation of large number of embryonic bodies [6–9]. However, this suspension culture is unable to be utilized for the suspension culture of anchorage-dependent cells including mesenchymal stem cells (MSCs) and other somatic cells. Thus, microcarrier-based suspension culture is a promising method for the large-scale culture of anchorage-dependent cells, because the method allows cell to adhere and proliferate on the surface of synthetic microbead (microcarrier) in stirred suspension [4,10–13]. In an industrial cell culture for producing the broad range of pharmaceutical recombinant proteins (*e.g.* vaccines and antibodies), this culture method has already been employed for expanding a large number of cells [11,12]. Since microcarrier surface provides a large surface area-to-volume ratio compared to two-dimensional planar surface, microcarrier culture can give the maximum achievable cell density. Actually, this microcarrier approach is also applied to the large-scale culture of chondrocytes [14], osteoblasts [15], bone marrow-derived MSCs [16,17], and ESC-derived cells [18], indicating that the development of novel microcarriers and their surface chemistry would contribute to the further progress of regenerative medicine.

To date, various synthetic beads consisting of dextran, glass, and poly(styrene) with a diameter range of 50–300 μm have been developed as a microcarrier [4,12,13]. However, most of these microcarriers are designed and optimized for obtaining a cell-derived biological product, repeated trypsinization is required in the process of passaging culture to harvest adhered cells from the surface of beads. This proteolytic enzyme treatment gives the

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degradations of plasma membrane proteins as well as deposited extracellular matrix (ECM), leading to the reduction of cell viability [19–21]. For the application of microcarrier culture to large-scale culture of therapeutic cells, the development of alternative microcarriers enabling non-invasive cell harvest without proteolytic enzymes and providing intact cells would be an advantageous candidate.

Worth noticing in this regard is the temperature-responsive poly(*N*-isopropylacrylamide) (PIPAAm)-grafted surface [22,23]. PIPAAm can be soluble in aqueous media below its lower critical solution temperature (LCST) around 32 °C. However, once temperature increase above LCST, PIPAAm dehydrates, and its conformation changes from random coil to globular. Our laboratories develop a temperature-responsive PIPAAm-grafted cell culture surface [19–23]. In synchrony with the temperature-dependent hydrophilic/hydrophobic property change of grafted PIPAAm, cell attachment and detachment can be regulated on this surface by only altering temperature without proteolytic enzyme treatment, *viz*, the adhered cells can be harvested by temperature reduction. Additionally, the complicated plasma membrane structure and secreted ECM are preserved in the process of thermally-induced cell detachment, meaning that this cell harvest method is non-invasive for cultured cells [19–21]. Thus, the application of temperature-responsive surface to the surface design of microcarrier is a promising approach for achieving both efficient large-scale cell culture and subsequent non-invasive cell harvest in a suspension culture system (Fig. 1).

In this study, temperature-responsive PIPAAm-grafted microcarriers were synthesized by the surface-initiated atom transfer polymerization (SI-ATRP) of IPAAm using chloromethylated poly(styrene) (CMPS) beads. Although a cell adhesion and detachment behavior on PIPAAm-grafted planar surface is extensively studied, that on PIPAAm-grafted spherical bead surface are never studied yet. Thus, the effect of physicochemical characteristics of PIPAAm-grafted beads including the grafted amount of PIPAAm and the diameter of bead on cell adhesion, proliferation in suspension culture, and thermally-induced detachment were investigated for achieving an optimal large-scale cultivation and subsequent thermally-induced cell harvest.

## 2. Materials and methods

### 2.1. Materials

Chloromethylated poly(styrene) (CMPS) beads with various mesh sizes were obtained from Aldrich (Milwaukee, WI, USA) (50–100 mesh) and Tokyo Chemical Industry (Tokyo, Japan) (100–200 mesh and 200–400 mesh). The average diameter, coefficient of variation, specific surface area, and chlorine content of the beads were summarized in Table 1. *N*-Isopropylacrylamide (IPAAm) was kindly provided from Kohjin (Tokyo, Japan) and purified by recrystallization from *n*-hexane. Tris[(2-dimethylamino)ethyl]amine ($Me_6$TREN) was synthesized according to the previously described procedure and purified by distillation under reduced pressure [24]. Copper(I) chloride (CuCl), copper(II) dichloride ($CuCl_2$) were obtained from Aldrich and used as received. α-Chloro-*p*-xylene and anhydrous 2-propanol were obtained from Wako Pure Chemical Industries (Osaka, Japan) and used as received. Ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA, disodium salt and dihydrate) was obtained from Dojindo Laboratories (Kumamoto, Japan). Milli-Q water used in this

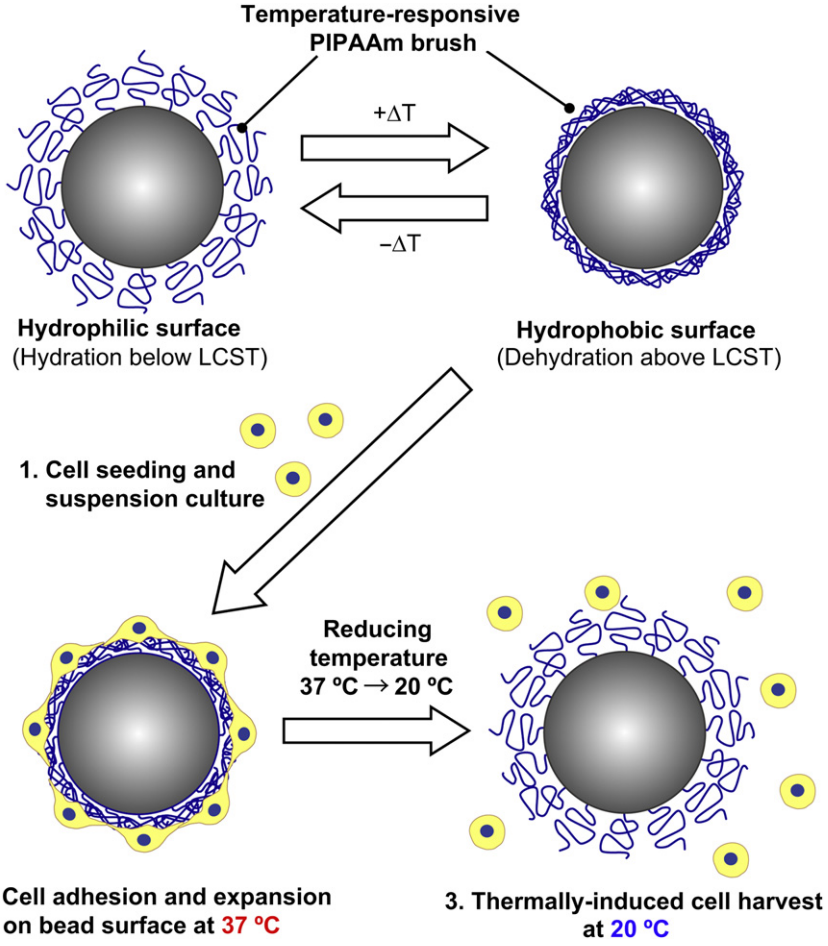


**Fig. 1.** Schematic illustration of PIPAAm-grafted microcarriers exhibiting a temperature-dependent hydrophilicity/hydrophobicity surface property change for cell cultivation and subsequent thermally-induced harvest.