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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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