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Continuous harvest of stem cells via partial detachment from thermoresponsive nanobrush surfaces

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ABSTRACT

Stem cell culture is typically based on batch-type culture, which is laborious and expensive. Here, we propose a continuous harvest method for stem cells cultured on thermoresponsive nanobrush surfaces. In this method, stem cells are partially detached from the nanobrush surface by reducing the temperature of the culture medium below the critical solution temperature needed for thermoresponse. The detached stem cells are harvested by exchange into fresh culture medium. Following this, the remaining cells are continuously cultured by expansion in fresh culture medium at 37 °C. Thermoresponsive nanobrush surfaces were prepared by coating block copolymers containing polystyrene (for hydrophobic anchoring onto culture dishes) with three types of polymers: (a) polyacrylic acid with cell-binding oligopeptides, (b) thermoresponsive poly-N-isopropylacrylamide, and (c) hydrophilic poly(ethyleneglycol) methacrylate. The optimal coating durations and compositions for these copolymers to facilitate adequate attachment and detachment of human adipose-derived stem cells (hADSCs) and embryonic stem cells (hESCs) were determined. hADSCs and hESCs were continuously harvested for 5 and 3 cycles, respectively, via the partial detachment of cells from thermoresponsive nanobrush surfaces.

1. Introduction

Stem cells, including human adult stem cells (hASCs), human embryonic stem cells (hESCs), and induced pluripotent stem cells (hiPSCs), are attractive reagents for regenerative medicine, translational medicine, and drug discovery [1–6]. hESCs and hiPSCs can provide a potentially unlimited cell source for cell therapy. However, hESCs and hiPSCs must be cultured under specific environmental conditions [7,8], such as overlaid on mouse embryonic fibroblasts, Matrigel, specific biomaterials immobilized by extracellular matrix (ECM) [9–15] and ECM-derived oligopeptides [16–19], or specific hydrogels [20,21]. Such conditions are required

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to maintain hESC and hiPSC pluripotency [22–24] because the easy differentiability of these cells precludes their culture on conventional culture dishes and microcarriers, although it is possible to culture hASCs on these materials. Therefore, the expensive and laborious cell culture processes required for hESCs and hiPSCs obstruct their use in regenerative and translational medicine. The majority of the expense associated with stem cell culture is derived from the use of disposable culture dishes and microcarriers, a requirement of the batch-type culture processes used with these cells. If a continuous cell culture system could be developed for the expansion of hASCs, hESCs, and hiPSCs (Fig. 1A), the cost of their culture would dramatically decrease, and stem cell therapies would advance significantly.

Stem cell culture processes have three steps: (1) cell seeding and attachment to a surface, (2) cell expansion, and (3) cell detachment. To obtain large numbers of stem cells for cell therapy, these steps must be followed repeatedly. In most cases, enzymes, such as trypsin for hASCs and dispase or accutase for hESCs and hiPSCs, are used to detach cells from cell culture dishes or microcarriers. As an alternative, culturing stem cells on a thermoresponsive nanobrush surface (i.e., polymer brush) would avoid the need for enzymes and simplify the cell detachment process by bypassing the need for centrifugation [25–33]. However, to accomplish this, biomaterials possessing thermoresponsive characteristics that maintain hESC and hiPSC pluripotency while facilitating cell detachment must be developed [25,28,29]. The detachment of hESCs and/or hiPSCs from thermoresponsive surfaces by decreasing the culture temperature from 37 °C to 4–20 °C has not yet been reported [25]. This is probably because hESCs and/or hiPSCs cannot proliferate on thermoresponsive surfaces while retaining their pluripotency unless specific binding sites on the cell surfaces are immobilized.

The thermoresponsive and non-thermoresponsive dishes and microcarriers used in conventional stem cell culture are disposable. To harvest large numbers of stem cells, the cells must be seeded

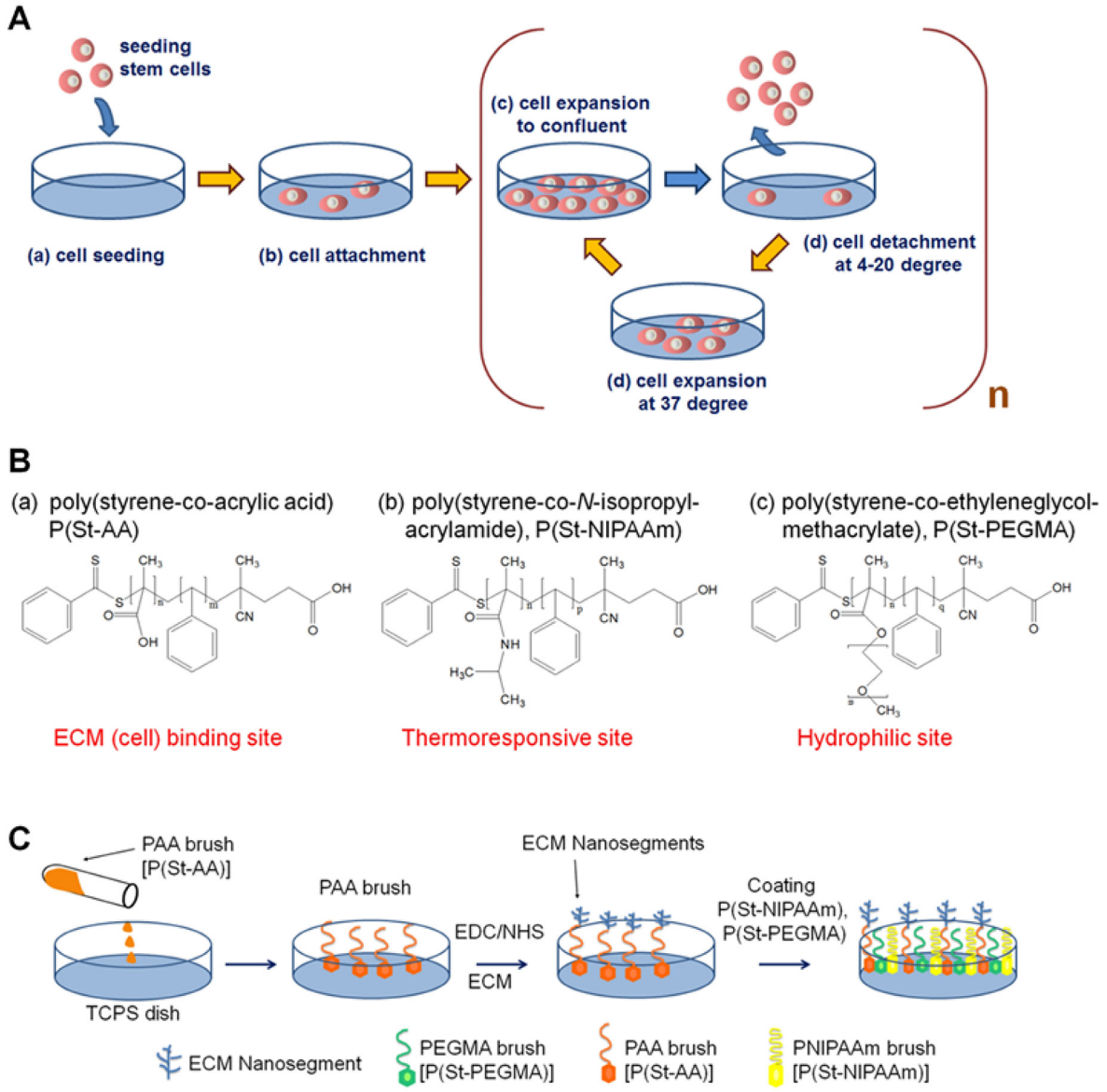

Fig. 1. Preparation of a thermoresponsive nanobrush surface. (A) Concept of continuous stem cell harvest. (B) Chemical structures of poly(styrene-co-acrylic acid) (P[St-AA]), poly(styrene-co-N-isopropylacrylamide) (P[St-NIPAAm]), and poly(styrene-co-ethylene glycol) methacrylate (P[St-PEGMA]). (C) Preparation of a thermoresponsive nanobrush surface by coating a dish with P[St-AA] grafted with oligoVN, P[St-NIPAAm], and P[St-PEGMA].