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Comb-type grafted poly(*N*-isopropylacrylamide) gel modified surfaces for rapid detachment of cell sheet

Zhonglan Tang, Yoshikatsu Akiyama, Masayuki Yamato, Teruo Okano*

Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University (TWIns), 8-1 Kawadacho, Shinjuku, Tokyo 162-8666, Japan

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ABSTRACT

A comb-type grafted poly(*N*-isopropylacrylamide) (PIPAAm) gel modified surface was newly developed for providing a rapid cell sheet recovery for tissue engineering. PIPAAm macromonomer was prepared by the etherification reaction of the hydroxyl terminal moieties of PIPAAm with acryloyl chloride, followed by the radical telomerization reaction of *N*-isopropylacrylamide (IPAAm) monomer using 2-mercaptoethanol as a chain transfer agent. Solution containing IPAAm monomer and PIPAAm macromonomer was spread on the surface of tissue culture polystyrene (TCPS), and then the surface was subjected to electron beam irradiation for grafting the monomer and macromonomer on the surfaces, resulting in comb-type grafted PIPAAm gel modified TCPS (GG-TCPS). Besides the difference of the amount of the modified PIPAAm, no distinct difference was found between the properties of GG-TCPSs and normal-type PIPAAm gel modified TCPS (NG-TCPS) through XPS, AFM and a contact angle measurement. At 37 °C, bovine aortic endothelial cells (BAECs) were well adhered and spread on GG-TCPS as well as NG-TCPS regardless of the macromonomer concentration. By lowering temperature to 20 °C, BAECs detached themselves more rapidly from GG-TCPS compared with NG-TCPS. Upon lowering temperature, the grafted polymer was speculated to accelerate the hydration of modified PIPAAm gel, resulting in a rapid cell sheet detachment.

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1. Introduction

Poly(*N*-isopropylacrylamide) (PIPAAm) is a well-known intelligent polymer exhibiting temperature-responsive soluble/insoluble changes in aqueous solution below and above lower critical solution temperature (LCST), 32 °C [1]. Our group has successfully developed cell culture surfaces by introducing 20 nm of ultrathin PIPAAm layer on tissue culture polystyrene (TCPS) surfaces by electron beams irradiation [2–6]. Due to the dehydration of the introduced PIPAAm chains, the PIPAAm gel modified surfaces showed a strong hydrophobic property that allows cells to adhered and grow confluently on the surfaces at 37 °C. By lowering temperature below the LCST, the cultured cells can be harvested as an intact cell sheet because of altering the property of surface from hydrophobic to hdyrophilic due to the hydration change of the PIPAAm chains [6]. Without using proteolytic enzyme that may affect cellular structure, the harvested cell sheet maintains the cell–cell junctions and deposited extracellular matrix (ECM) underneath of the cell sheet. Furthermore, the harvested cells, which connected each other with ECM, can preserve their intrinsic physiological functions and can express their bioactivities, which is similar to that of cells in living tissue [7]. Various kinds of cell sheets such as epidermal keratinocytes [8], vascular endothelial cells [9], corneal epithelial cells [10], oral mucosal epithelial cells [11], renal epithelial cells [12,13], periodontal ligaments [14,15], hepatocytes [16], and cardiomyocytes [17,18] have been successfully fabricated with the temperature-responsive cell culture surfaces. In particular, corneal epithelial cell sheets and oral mucosal epithelial cell sheets have been clinically applied to human corneal reconstruction [10,11]. This new approach is called "cell sheet engineering" [4].

Cultured cells could detach from the PIPAAm modified surface spontaneously, however, the detachment process is a slow process and takes 20–30 min or more time in the static condition. Probably, this is because that the detachment of cell sheet proceeds gradually from the sheet periphery toward the central part, being affected by the hydration of modified PIPAAm layer. Moreover, the activity of cellular metabolism is dependent on the species of cultured cells and culture conditions, having an influence on following cell detachment process [19]. Provided that cell sheet engineering are exploited in clinical application, rapid cell sheet detachment should be desired for following cell sheet transplantation and construction of tissue or organ, which is comprising of layered cell sheets.

* Corresponding author. Tel.: +81 3 5367 9945x6201; fax: +81 3 3359 6046.
E-mail address: tokano@abmes.twmu.ac.jp (T. Okano).

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Consequently, the rapid detachment leads to reduce patient's burden. Thereby, temperature-responsive cell culture surfaces enabling rapid cell sheet detachment is an important tool. The hydration of PIPAAm chains was accelerated to attain rapid cell detachment by using porous membrane and/or introducing hydrophilic component such as polyethylene glycol (PEG) or poly (2-carboxylisopropylacrylamide) (PCIPAAm) moiety that was incorporated into PIPAAm gel [20–22]. In the former case, water penetration through the porous membrane to basal side of cell sheet accelerates the hydration of PIPAAm at the interface between the surface and the cell sheet, achieving a rapid detachment [20,21]. In the latter case, small amount of hydrophilic comonomer component dos not affect cell adhesion and growth so much, and accelerate the diffusion of water molecules by the incorporated hydrophilic component. Cell detachment as well as the hydration of PIPAAm gel containing the hydrophilic component is significantly accelerated [22]. Our group has reported comb-type grafted PIPAAm hydrogels, which contain PIPAAm graft chains with a freely mobile end, and shows unique behaviors for the enhancement of the rate of swelling–deswelling of the hydrogel in response to temperature change [23–25]. Above LCST, the graft chains dehydrate and aggregate faster than PIPAAm cross-linked network due to the freely mobile end of the graft polymer. As a result, PIPAAm cross-linked network shrinks due to hydrophobic clusters formed inside the polymer network, and water is rapidly excluded from the gel (Fig. 1A) [23]. In contrast, below LCST, the graft polymer chains are hydrated faster, inducing the rapid hydration and swelling of PIPAAm gel (Fig. 1B) [25].

In this study, the characteristic of comb-type grafted PIPAAm gel was applied to temperature-responsive cell culture surfaces. Namely, PIPAAm macromonomer component were introduced into PIPAAm cross-linked network, and comb-type grafted PIPAAm gel on TCPS was newly developed to attain more rapid cell sheet recovery from temperature-responsive cell culture surfaces (Fig. 1C).

2. Materials and methods

2.1. Materials

N-Isopropylacrylamide (IPAAm) was kindly provided by Kohjin (Tokyo, Japan) and purified by recrystallization twice from *n*-hexane. 2-Mercaptoethanol (2-ME) (Wako Pure Chemical Industries, Osaka), acryloyl chloride (Wako), tetrahydrofuran (THF) (Wako) without stabilizer and benzoyl peroxide (BPO) (Sigma, St Louis, MO, USA) were used as received. *N*,*N*-Dimethylformamide (DMF) (Wako) was distilled under reduced pressure (b.p. 45 °C/20 mmHg). Tissue culture polystyrene (TCPS) dishes (35-mm in diameter, Falcon 3001) were purchased from BD Biosciences (Billerica, MA). Water used in this study was purified by a Milli-Q water purification system (Milli-Q Synthesis A10) (Millipore, Billerica, MA) unless otherwise mentioned.

2.2. Preparation of PIPAAm macromonomer

PIPAAm macromonomer was prepared according to a previously reported procedure [26,27]. PIPAAm polymer with a terminal hydroxyl end group (PIPAAm–OH) was synthesized by the radical telomerization of IPAAm monomer using 2-ME as a chain transfer agent. As shown in Fig. 2A, specific amounts of IPAAm, 2-ME, and BPO as an initiator were dissolved in 100 mL of DMF in a round-bottomed flask connected with a two-way stopcock. The mixture was degassed by freeze-pump-thaw cycles for three times. The radical polymerization was carried out at 70 °C for 1 h. After being concentrated by DMF evaporation, the reactant was poured into an excess amount of diethyl ether to precipitate the product, PIPAAm–OH. The precipitated compound was collected over a filter and dried under vacuum. Obtained polymer was further purified by repeated precipitation in diethyl ether from DMF.

PIPAAm macromonomer was prepared by the reaction of hydroxyl group of PIPAAm–OH with acryloyl chloride via esterification reaction (Fig. 2B). The purified

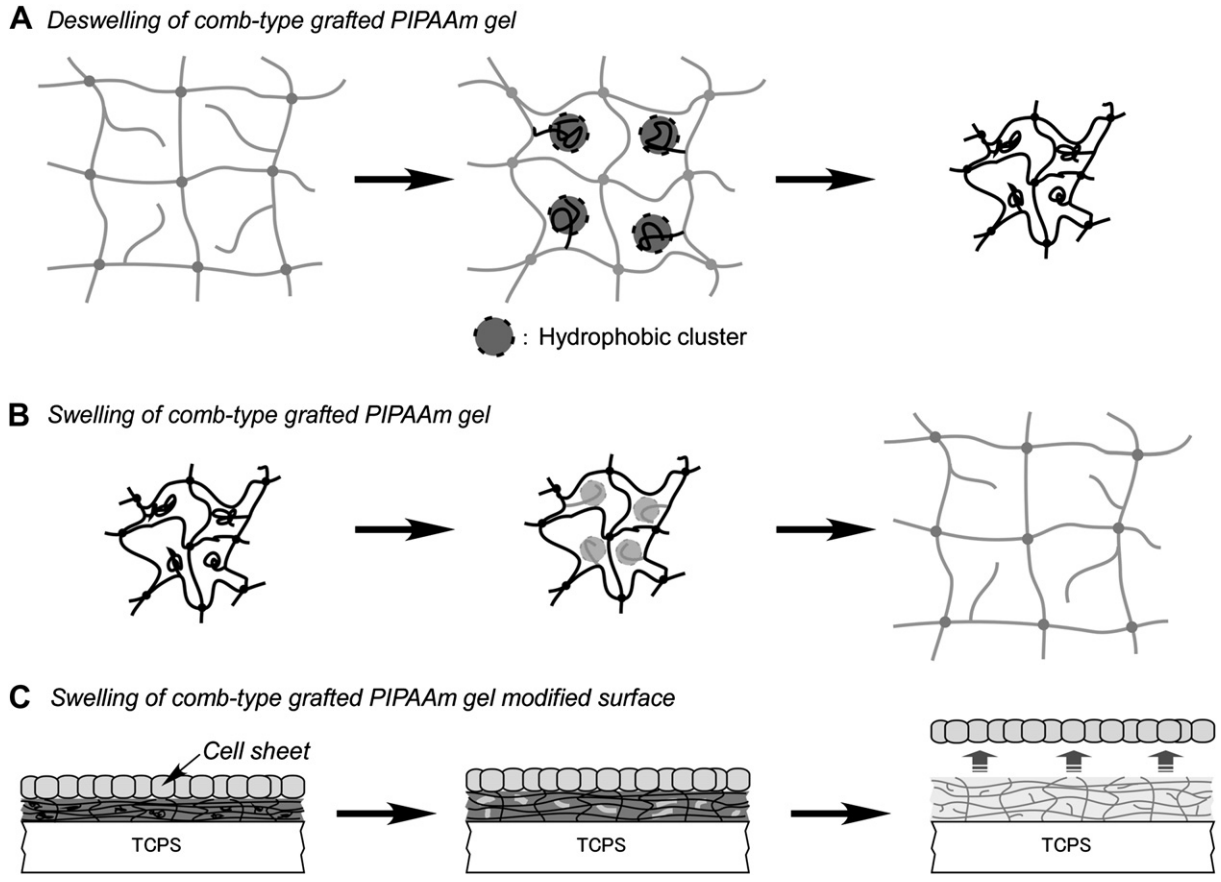

Fig. 1. Schematic drawing of deswelling (A) and swelling (B) of comb-type grafted PIPAAm gel and the swelling of comb-type grafted PIPAAm gel modified surface (C).

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