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Engineering cell de-adhesion dynamics on thermoresponsive poly(*N*-isopropylacrylamide)

Beiyi Chen [a], F.J. Xu [b,c], Ning Fang [a], K.G. Neoh [b,*], E.T. Kang [b], Wei Ning Chen [a], Vincent Chan [a,*]

[a] *Center of Biotechnology, School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 639798, Singapore*
[b] *Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore 119260, Singapore*
[c] *Division of Bioengineering, National University of Singapore, Singapore 119260, Singapore*

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Abstract

Poly(*N*-isopropylacrylamide) (PIPAAm) has been demonstrated as an effective thermoresponsive polymer for non-invasive cell regeneration/recovery. However, little is known about the intricate relationship between the biophysical response of cells and physiochemical properties of PIPAAm during cell recovery. In this study, the de-adhesion kinetics of smooth muscle cell (SMC) on PIPAAm surfaces is probed with unique biophysical techniques. Water-immersion atomic force microscope (AFM) first showed that the nanotopology of PIPAAm surfaces is dependent on the polymerization time and collagen coating. It is found that the initial rate of cell de-adhesion increases with the increase in polymerization time. Moreover, the degree of cell deformation (a/R) and average adhesion energy are reduced with the increase of grafted PIPAAm density during 40 min of cell de-adhesion. It has been shown that collagen coating regulates cell adhesion on biomaterial surface. Interestingly, lower collagen density on PIPAAm leads to higher adhesion energy per cell during the initial 20 min compared with as-prepared PIPAAm, while the initial rate of cell de-adhesion remains unchanged. In contrast, higher collagen density leads to 50% reduction in the initial rate of cell de-adhesion and higher adhesion energy per cell during the entire 90 min. Furthermore, immunostaining of actin provides supporting evidence that the de-adhesion kinetics is correlated with the cytoskeleton transformation during cell de-adhesion below the lower solution critical temperature (LCST).

Keywords: De-adhesion; Kinetics; SMC; Poly(*N*-isopropylacrylamide); Collagen

1. Introduction

Thermoresponsive polymer (TRP) has emerged as a promising class of biomaterial for the regeneration of various cells such as hepatocytes, endothelial cells, urothelial cells and fibroblasts [1,2]. The application of TRP for the non-invasive recovery of cells/tissues is brought about by its acute switching of physiochemical properties across the lower critical solution temperature (LCST). In particular, this technique eliminates the need for proteolytic enzymes or physical scraping for recovering cells from a tissue culture dish, and preserves the intracellular junctions as well as native tissue organization.

One of the earliest members of the TRP family, poly(*N*-isopropylacrylamide) (PIPAAm), forms a hydrogel which is transformed from a swelling state to a collapsed state across its LCST [3]. Moreover, the LCST of PIPAAm can be engineered with the design of block copolymers containing PIPAAm and other polymers. To date, PIPAAm and its copolymers have been widely exploited for applications in bioseparation, drug delivery and other thermoresponsive devices [4,5]. Okano and co-workers have

* Corresponding authors. Tel.: +65 67904040 (V. Chan).
E-mail addresses: chenkg@nus.edu.sg (K.G. Neoh), mvchan@ntu.edu.sg (V. Chan).

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pioneered the chemical grafting of PIPAAm onto tissue culture polystyrene (TCPS) by electron beam irradiation [6]. Alternatively, PIPAAm films may also be prepared by physical adsorption of the polymer chains onto glass [7], plasma polymerization of *N*-isopropylacrylamide (IPAAm) onto TCPS [8], photografting [9], gamma radiation [10], plasma immobilization [11] and plasma-induced graft polymerization [12]. It has been shown that the acute increase of hydrophilicity on PIPAAm films with the reduction in temperature drives the detachment of a confluent cell layer. Moreover, the time required for complete cell sheet detachment from PIPAAm films is highly dependent on cell types [13] and physiochemical properties of TRP.

The biocompatibility of PIPAAm is less ideal than conventional TCPS for inducing strong cell adhesion, which is critical for effective tissue regeneration [14]. The biocompatibility of PIPAAm is improved by the incorporation of hydrophobic copolymer into the PIPAAm chain [15,16] or coating with extracellular matrix (ECM) proteins [17]. Collagen is the most abundant ECM protein found in mammalian tissues, forms a fibrous fiber with great tensile strength [18,19] and plays a key role in the physiological regulation of smooth muscle cell (SMC). It has also been shown that cell adhesion and migration on biomaterial surfaces are dependent on the concentration of adsorbed collagen [20]. SMC is an interesting model system for TRP because the non-invasive recovery of engineered tissues containing native SMC is a critical area of vascular tissue engineering [21].

The recent development of integrated biophysical techniques has elucidated the adhesion contact dynamics of cells on biomaterials during the initial cell seeding [22,23]. Recently, our unique approach has been successfully applied to probe the de-adhesion kinetics of single SMC from hydroxybutyl chitosan [24]. Moreover, our group has developed an improved two-step reaction to couple PIPAAm chains to silicon coverslips [25]. To the best of our knowledge, there is currently a lack of quantitative correlation between the physiochemical properties of PIPAAm films and the resulting de-adhesion kinetics of cells upon temperature reduction. In this study, the dynamics of SMC de-adhesion on PIPAAm surfaces under different polymerization times and collagen coating are probed with our unique biophysical techniques. Specifically, confocal reflectance interference contrast microscopy (C-RICM) in combination with phase contact microscopy determined key biophysical parameters of cell de-adhesion including initial de-adhesion rate, degree of deformation, average adhesion energy and adhesion energy per cell of SMCs. In addition, the cytoskeleton structure of SMC during the course of thermal-induced de-adhesion is detected by confocal fluorescence microscopy through immunostaining of actin. It is demonstrated that the de-adhesion dynamics and cytoskeleton transformation of SMC can be engineered by the polymerization time and collagen modification of PIPAAm surfaces. The elucidation of the kinetics of cell de-adhesion from different PIPAAm surfaces would likely provide important insights for designing highly tailored process for cell regenerations.

2. Materials and methods

2.1. Materials

High glucose Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), streptomycin, penicillin, 10× Trypsin–EDTA (0.5%) and 10× phosphate-buffered saline (PBS) (pH 7.4) were purchased from Gibco (Singapore). $CuCl_2$, CuCl, NIPAAm, 1,1,4,7,10,10-hexamethyl-triethylenetetramino, paraformaldehyde, Triton X-100, fluorescein isothiocyanate (FITC)–phalloidin and anti-vinculin–FITC-conjugated antibody were purchased from Sigma Chemical Pte. Ltd. (Singapore). Type I collagen was bought from Benton Dickinson (Singapore). The reagents were diluted with 1× PBS. Highly purified 18.2 MΩ water was obtained from water purification system (Sartorius, Germany). 4(Chloromethyl)phenyltrichlorosilane (97%) was obtained from Alfa Aesar (Singapore).

2.2. Preparation of PIPAAm surface

Glass coverslips were cleaned with piranha solution (30% H_2O_2 with 70% H_2SO_4) for 1 h, rinsed thoroughly with 18.2 MΩ water and then dried under vacuum at room temperature. The procedures for the preparation of PIPAAm-grafted surfaces using the atom transfer radical polymerizations (ATRP) reaction have been described elsewhere [25,26]. In brief, the glass coverslips were first immersed in 30 ml of chloroform followed by 0.5 ml of triethylamine and 2 ml of 4-(chloromethyl)phenyltrichlorosilane. After reaction for 24 h, the coverslips were washed thoroughly with acetone and kept in acetone for another 30 min to remove the unreacted silane. After the coverslips were dried in air, they were added to 15 ml of 0.23 g ml^{-1} NIPAAm solution. Then 20 mg of CuCl and 4 mg of $CuCl_2$ were added and the tube was degassed with argon for 20 min. Finally, 50 μl of 1,1,4,7,10,10-hexamethyl-triethylenetetramino was added to the mixture and the tube was sealed for the interfacial polymerization of NIPAAm. To terminate the polymerization, the samples were removed from the reaction mixture and washed sequentially with copious amounts of DMSO and double-distilled water, prior to being dried under reduced pressure. Throughout this report, NIPAAm surfaces obtained from different polymerization times of IPAAm are represented by the time following the polymer notation; for example, the sample with a polymerization time of 0.5 h is represented by PIPAAm-0.5 h.

2.3. Preparation of collagen-coated surface

The original collagen solution (2.9 mg ml^{-1} in 0.12 N HCl) was diluted to 0.1× (0.29 mg ml^{-1}) and 0.04× (0.12 mg ml^{-1}). A 200 μl quantity of solution at each

ID	Title	Pages
1832	Engineering cell de-adhesion dynamics on thermoresponsive poly(N-isopropylacrylamide)	12