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Poly(*N*-isopropylacrylamide) modified polydopamine as temperature-responsive surface for cultivation and harvest of mesenchymal stem cells

Jun Zhang and Ching-An Peng*

Department of Biological Engineering, University of Idaho, Moscow, ID 83844, USA

Abstract

A thermo-responsive surface was fabricated by depositing poly(*N*-isopropylacrylamide) (PNIPAAm) onto polydopamine coated cell culture substrata through free radical polymerization for the purpose of culturing and harvesting human mesenchymal stem cells (hMSCs). Human MSCs were cultured onto PNIPAAm-*g*-polydopamine coated surface and harvested by changing from physiological to ambient temperature. The produced PNIPAAm-*g*-polydopamine surface was characterized by atomic force microscopy, Fourier transform infrared spectroscopy, nuclear magnetic resonance, water contact angle measurement, differential scanning calorimetry, and cell culture studies. Our results revealed that hMSCs could be detached from PNIPAAm-*g*-polydopamine surface within 60 min after switching temperature from 37°C to room temperature. The detached hMSCs were able to proliferate on PNIPAAm-*g*-polydopamine coated surface for further growth and harvest.

Graphical abstract

A facile approach for the production of PNIPAAm-*g*-polydopamine film in cultivation and harvest of mesenchymal stem cells through LCST transition

1. Introduction

Poly(*N*-isopropylacrylamide) (PNIPAAm) is an extensively studied thermo-sensitive polymer with a lower critical solution temperature (LCST) of 32°C in aqueous solution.¹ In

Corresponding address: Ching-An Peng, Department of Biological Engineering, Engineering Physics Building 421, 875 Perimeter Drive MS 0904, Moscow, ID 83844-0904, Phone: 208-885-7461, capeng@uidaho.edu.

the past, PNIPAAm modified surfaces have been used to harvest high-density cell sheets without disrupting the extracellular matrix proteins.² This is because PNIPAAm is hydrophobic in nature at temperatures higher than 32°C but becomes hydrophilic as the temperature falls. Cells attach more easily on hydrophobic surfaces than on hydrophilic, therefore, when the temperature of the PNIPAAm surface is lowered, the cells can easily detach. Although using traditional proteolytic enzymes such as trypsin for cell harvesting is highly efficient, enzymatic treatment might cause disruption of the cell membrane and/or extracellular matrix (ECM) of the cells,³ and make the mesenchymal stem cells lose their differentiated phenotypes.⁴ Cell harvesting from PNIPAAm-grafted surface can avoid damage of the ECM.^{3, 5} The intact ECM allows the cell sheets to be used directly for transplantation.⁶ Mesenchymal stem cells (MSCs) have been extensively studied due to their self-renewal property and multi-differentiation potential for the field of tissue engineering and regenerative medicine.⁷ Due to tumor-tropic ability, MSCs have also been gaining importance in cancer therapy as cell carriers for delivering therapeutic agents.⁸ Treating MSCs with trypsin for an extended time period and after multiple passages could harm MSCs and affect their proliferation. Yang et al reported that MSCs harvested from PNIPAAm-modified surfaces have shown higher cell viability, better proliferation, and stronger differentiation than MSCs harvested by trypsinization after three cell passages.⁴

Various techniques have been used to fabricate PNIPAAm-modified surfaces for cell and tissue engineering purposes. These methods include electron beam irradiation, plasma polymerization, UV irradiation, and spin coating.^{9–12} Among all these methods, electron beam polymerization is the most employed method for producing PNIPAAm surfaces because the thickness of the polymer film can be carefully controlled to 15 – 20 nm, which is the optimized thickness for cell adhesion and temperature-controlled detachment.^{3, 13} In view of polydopamine film having been employed to serve as an excellent platform for further surface functionalization and biomolecule immobilization,^{14–16} this study intends to use polydopamine as the surface modification material to fabricate PNIPAAm surface by free radical polymerization for culturing and harvesting hMSCs. Polydopamine has recently drawn significant attention as a universal surface modification agent which can be prepared by simply immersing substrates into an aqueous dopamine solution under alkaline conditions.¹⁷ The polydopamine film is ultrathin but robust, and it can be used to modify the surfaces of a vast range of materials.

Although polydopamine/PNIPAAm mixed films have been reported by mixing aminated PNIPAAm into the dopamine solution prior to the film deposition,¹⁸ myoblast cells revealed less spreading feature than the pristine coatings after 24 h attachment. Moreover, no attempts were made to harvest cells with the fabricated polydopamine/PNIPAAm mixed films. In this study, a straightforward and cost-effective two-step method was used to produce PNIPAAm-*g*-polydopamine coated Petri dishes. Compared with other methods for producing PNIPAAm modified surfaces,^{9–12} this facile two-step approach involves merely the deposition of biocompatible polydopamine film and free radical polymerization, without the need of any special instrument. In the first step, dopamine was first tagged with allyl groups by conjugating with itaconic anhydride (ITA). In the conjugation process, ITA reacted with the amine group of dopamine, and the anhydride ring was opened by nucleophilic attack (Figure 1). In the second step, the allyl-functionalized polydopamine film was then coated

on the substratum by adding allylated dopamine into solution with slight basic pH. Through the allyl groups immobilized on the substratum, PNIPAAm was able to crosslink onto the surface by free radical polymerization.

The fabricated PNIPAAm-*g*-polydopamine substrata after characterization were used to culture and harvest hMSCs. The morphology of cell shape, attachment, and spreading on the PNIPAAm-*g*-polydopamine surface was essentially the same as the ones observed on bare and polydopamine coated culture dishes. Our results showed that within 60 min, majority of cultured hMSCs could be harvested from the PNIPAAm-*g*-polydopamine coated substrata by treating hMSCs with culture medium at ambient temperature.

2. Experimental Section

2.1. Materials

N-isopropylacrylamide (NIPAAm) was purchased from TCI (Tokyo, Japan). Dopamine hydrochloride, dimethyl sulfoxide-*d*₆ (DMSO-*d*₆) and penicillin-streptomycin was purchased from Sigma-Aldrich (St. Louis, MO, USA). Tris (hydroxymethyl) aminomethane (Tris base), sodium hydroxide (NaOH), 0.25% trypsin/EDTA solution, Minimum Essential Medium Alpha Medium (αMEM), Dulbecco's Modified Eagle's Medium (DMEM) and L-glutamine were purchased from Fisher Scientific (Waltham, MA, USA). *N*-isopropylacrylamide (NIPAAm) was purchased from TCI America (Portland, OR, USA). Ammonium persulfate (APS) was purchased from J.T. Baker (Philipsburg, NJ, USA). Itaconic anhydride (ITA), and 99% N,N,N',N'-tetramethylethylenediamine (TEMED) were purchased from Alfa Aesar (Ward Hill, MA, USA). Fetal bovine serum (FBS) was purchased from Atlanta Biologicals (Lawrenceville, GA, USA). Human mesenchymal stem cells were obtained from Texas A&M Health Science Center (College Station, TX, USA).

2.2. Preparation of PNIPAAm-*g*-polydopamine modified substratum

In order to modify the polydopamine film with allyl functional group, dopamine was first conjugated with itaconic anhydride (ITA) to form acylated dopamine before the polymerization process. Briefly, 0.04 g of dopamine and 0.04 g of ITA was dissolved in 20 mL of deionized (DI) water. The reaction mixture was stirred for 2 h. The pH of the solution was adjusted to 8.5 by adding Tris base to the solution. A 35-mm Petri dish was immersed in the mixture and secured at the bottom of the beaker. The beaker was then placed in an orbital incubator (MaxQ 4000; Thermo Fisher Scientific, Waltham, MA, USA) shaken at 100 rpm for overnight. The polydopamine modified substrate was cleaned by sonication in DI water for 10 min.

After the substrate surface was functionalized with allyl groups, PNIPAAm was deposited on the surface by free radical polymerization in the presence of APS and TEMED. Briefly, 15 mL of 0.5 M NIPAAm solution was prepared in DI water, and the polydopamine modified Petri dish was immersed in the NIPAAm solution. 0.3 g of APS and 10 μL of TEMED were added to the NIPAAm solution, and the mixture was stirred at ambient temperature for 2 h. The PNIPAAm-*g*-polydopamine coated Petri dish was collected and repeatedly rinsed by DI water.

2.3. Characterization of PNIPAAm-*g*-polydopamine surface

To check whether PNIPAAm has been grafted onto the polydopamine surface, the surface topography of polydopamine and PNIPAAm-*g*-polydopamine films were compared. The tapping mode of atomic force microscopy (AFM) (Dimension 3000; Veeco Instruments, Plainview, NY, USA) was used, and both topographical and 3D images were acquired by Nanoscope software (Veeco Instruments). Sectional analysis was used to estimate the thickness of the film. The images were captured at a scan size of 5 μm .

The chemical structure of the polydopamine film and PNIPAAm-*g*-polydopamine film was characterized by Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR). FTIR spectrum was recorded by an ATR/FTIR Spectrometer (Spectrum One; Perkin Elmer, Waltham, MA, USA). ^1H NMR spectra were obtained by an NMR spectrometer (INOVA 400 MHz; Varian, Palo Alto, CA, USA). First, the PNIPAAm-*g*-polydopamine film was dissolved in 0.1 N NaOH. Then, 50 μL of the dissolved film was mixed with 550 μL DMSO- d_6 to obtain NMR spectrum.

The LCST of the dissolved PNIPAAm-*g*-polydopamine film was characterized by differential scanning calorimetry (DSC 2920; TA Instruments, New Castle, DE, USA). DSC thermogram was obtained with a heating rate of 10°C per min under N_2 purge. The cloud point temperature of the dissolved film, following the method described by Jain et al,¹⁹ was determined by monitoring UV-Vis transmittance at 563 nm (Spectramax M2e microplate reader, Molecular Devices, Sunnyvale, CA, USA) in the temperature range of 25 to 37°C . The wettability of the PNIPAAm-*g*-polydopamine coated surface was characterized by water contact angle measurements on a goniometer (Krüss G10; KRÜSS GmbH, Hamburg, German). A clean bare Petri dish and a polydopamine coated petri dish were used as controls during the experiment. Here, a water droplet of 1 μL was dripped onto the control Petri dishes or the PNIPAAm-*g*-polydopamine coated Petri dish. The contact angles of the substrate surface were measured accordingly at ambient temperature and 40°C .

2.4. Culturing and harvesting hMSCs on PNIPAAm-*g*-polydopamine surface

The PNIPAAm-*g*-polydopamine coated Petri dish was placed in a biological safety cabinet (1300 Series Class II, Thermo Fisher Scientific, Waltham, MA, USA) under UV irradiation for 1 h, and sterilized with 70% ethanol for 30 min. hMSCs were inoculated onto the PNIPAAm modified Petri dish at 1.0×10^4 cells/ cm^2 and incubated at 37°C in a 5% CO_2 incubator (Forma 310; Thermo Fisher Scientific, Waltham, MA, USA). The cells were cultured with αMEM supplemented with 16.5% FBS, 1% penicillin-streptomycin and 2 mM L-glutamine. Once the cells reached confluency, cells were harvested by lowering the temperature from 37°C to ambient temperature by replacing culture medium with αMEM kept in the room temperature. The cells were observed for 60 min after the temperature change, and phase contrast images were taken every 20 min with Leica DMI8 microscope equipped with Leica DFC360 FX camera (Leica Microsystems, Wetzlar, Germany). At 20, 40, and 60 min, hMSCs were allowed to detach by giving the cells some gentle pipetting force. All images were processed by Leica Application Suite V3 software. Detached cells were counted using a hemocytometer. After 60 min, the cells not detached from the surface were harvested by treatment of 0.25% trypsin/EDTA, and the cell numbers were counted

again. The numbers of cells detached by temperature switch and trypsin treatment were added up as the total cell number. The percentage of cell detachment was plotted against time from 0 to 60 min.

hMSCs collected from PNIPAAm-*g*-polydopamine coated Petri dishes by temperature switch were centrifuged and resuspended in α MEM medium. The collected cells were inoculated on freshly prepared PNIPAAm-*g*-polydopamine coated Petri dishes at 10^3 cells/cm² and incubated at the aforementioned conditions. The cell numbers were counted every 3 days using a hemocytometer. Phase contrast images were taken with Leica DMI8 microscope equipped with Leica DFC360 FX camera.

3. Results and Discussion

3.1. Surface topography of PNIPAAm-*g*-polydopamine film

Thermo-responsive polymer PNIPAAm has been widely used in tissue and cell sheet engineering. Various methods have been developed to produce PNIPAAm surfaces for culturing and harvesting hMSCs.^{4, 11, 20, 21} Human MSCs cultivated and harvested from PNIPAAm surfaces have shown higher cell viability, and retaining their self-renewal and multipotential activities after detaching from temperature-responsive surfaces, unlike trypsinized hMSCs with ECM proteins being damaged.⁶ PNIPAAm surface has been produced onto polydopamine coated surface using surface-initiated atom transfer radical polymerization (SI-ATRP) for making controlled release of fertilizers.²² Although the thickness of the PNIPAAm surface can be well controlled using SI-ATRP,^{23, 24} the metal catalyst used in SI-ATRP is difficult to remove. This may cause cell toxicity problem during the cultivation of fastidious hMSCs. The free radical polymerization approach used here for the formation of PNIPAAm-*g*-polydopamine has no concern of leaving toxic residues for hMSC culture. Polydopamine/PNIPAAm mixed film was prepared by co-depositing polydopamine with amino-PNIPAAm.¹⁸ The reactive quinones of the polydopamine react with nucleophilic amines via the Michael-type addition and/or Schiff base reaction. Generally, aliphatic amines favor the Schiff base reaction which could be affected by the chain length of the chemical incorporating the primary amine.²⁵ A longer chain leads to a decrease in the basicity of the primary amine, which results in a lower reaction rate.²⁵ The long aliphatic chain of the reported aminated PNIPAAm (MW of 2,500)¹⁸ probably decreased the coupling of amine-ended PNIPAAm with the quinone groups of polydopamine. This could lead to less detachment of the myoblast cells via the temperature switch. Unlike that, the polydopamine film reported here was modified with allyl groups through a simple anhydride ring-opening reaction. Our PNIPAAm film was deposited on the allyl-functionalized polydopamine surface via surface-initiated free radical polymerization. The synthesis of PNIPAAm from the pre-deposited allylated polydopamine assured the temperature-responsive PNIPAAm was on the top layer of the fabricated surface for efficient culture and harvest of hMSCs.

The surface topography of the synthesized PNIPAAm-*g*-polydopamine surface was investigated by AFM. The surface of polydopamine coated Petri dish was fairly smooth (Figure 2A). During pH-induced polymerization of dopamine, dopamine self-polymerized and formed nano-aggregates in the bulk solution, and those nanoparticles clustered randomly

and were deposited onto the substrate surface.²⁶ Cross sectional analysis showed the deposition of a uniformly distributed polydopamine film onto the Petri dish. However, after grafting PNIPAAm by free radical polymerization, the PNIPAAm polymers were randomly deposited onto the substrate surface, with the height of the polymer aggregation from 50 to 100 nm. Although the height of the PNIPAAm-*g*-polydopamine polymer aggregate exceeded the optimum thickness (i.e., 15 – 20 nm) for temperature controlled cell harvesting,³ hMSCs were able to proliferate on the PNIPAAm-*g*-polydopamine modified Petri dish and be harvested through temperature switch.

3.2. Chemical structure of PNIPAAm-*g*-polydopamine film

The chemical structure of PNIPAAm-*g*-polydopamine film was characterized by FTIR spectroscopy. Figure 3 shows the FTIR spectra of PNIPAAm, polydopamine film and PNIPAAm-*g*-polydopamine film. In the spectrum of freeze-dried PNIPAAm powder, an absorption peak was observed at 3300 cm⁻¹ due to the N-H stretching.²⁷ Also, three other absorption peaks at 2970, 2960, and 2900 cm⁻¹ were observed next to the broad band. These peaks were due to the -CH₃ asymmetric stretching of PNIPAAm.²⁸ Three more absorption peaks were observed at 1620, 1550, and 1460 cm⁻¹. The peaks at 1620 and 1550 cm⁻¹ were the typical amide I and amide II stretching from PNIPAAm.²⁷ The peak at 1460 cm⁻¹ is the C-H stretching band. The characteristic peaks of PNIPAAm were all present in the spectrum of PNIPAAm-*g*-polydopamine. In the acquired spectrum of polydopamine film, a broad band was observed from 2500 to 3600 cm⁻¹, due to O-H stretching. Two distinctive peaks were observed at 1600 and 1500 cm⁻¹. These two peaks are the typical absorption peaks for indole structure.²⁹ However, these two peaks did not show up in the spectrum of PNIPAAm-*g*-polydopamine, likely because the chemical composition of the PNIPAAm-*g*-polydopamine film consisted mainly of PNIPAAm. Finally, in the spectrum of PNIPAAm-*g*-polydopamine, a narrow absorption peak was observed at 3280 cm⁻¹ owing to the N-H stretching. This was consistent with the N-H peak observed in freeze-dried PNIPAAm spectrum. The peaks at 2970, 2960, and 2900 cm⁻¹ were consistent with the -CH₃ asymmetric stretching in the PNIPAAm spectrum. The absorption peaks observed at 1620, 1550, and 1460 cm⁻¹ were the amide I and amide II peaks as well as C-H stretching peaks. The peaks of polydopamine were relatively weak compared to those of PNIPAAm because in PNIPAAm-*g*-polydopamine, the mass of PNIPAAm was much higher than the mass of polydopamine. In short, judging from the obtained FTIR spectra, it is confirmed that PNIPAAm film was established on the substrate surface via surface-initiated free radical polymerization from the surface pre-coated with polydopamine.

Figure 4 shows the ¹H NMR spectrum of PNIPAAm-*g*-polydopamine film which was first dissolved in NaOH solution (pH > 11) and then mixed with DMSO-d₆. Chemical shifts at δ = 5.50 to 5.53 ppm, 5.99 to 6.17 ppm, 7.04 ppm and 7.97 ppm were assigned to protons in polydopamine. The structure of polydopamine film contains both 5,6-dihydroxyindole and 5,6-indolequinone.³⁰ Both structures have protons in the benzene ring and pyrrole ring. Multiple peaks observed at 6 to 6.2 ppm were due to the chemical shift of protons in the benzene ring. The protons of the pyrrole ring showed up at the chemical shift of 5.5 ppm. Characteristic peaks associated with PNIPAAm include δ = 1.03 ppm, 1.88 ppm, 2.20 ppm, 3.6 to 3.9 ppm. The peak present at 1 ppm was due to the protons of the methyl groups. The

peaks at around 2 ppm were due to the methylene protons, and the peaks at 3.8 ppm were due to the protons of *N*-isopropyl groups. Finally, the chemical shift at 7.2 ppm was assigned to the amide protons. The peak intensity for PNIPAAm was much higher than those peaks for polydopamine, because the corresponding mass of PNIPAAm was much higher than polydopamine.

3.3. Water contact angle and LCST of PNIPAAm-*g*-polydopamine film

The LCST for PNIPAAm-*g*-polydopamine polymer was determined by DSC thermogram. Figure 5A shows the enthalpy transition as a function of temperature for the PNIPAAm-*g*-polydopamine polymer film dissolved in 0.1 N NaOH. PNIPAAm-*g*-polydopamine showed an endothermic peak at 30.4°C which is lower than the reported LCST (i.e., 32°C) of PNIPAAm film.¹ It is speculated that the incorporation of a relatively hydrophobic copolymer to PNIPAAm led to the reduction of the LCST.^{31, 32} In fact, the higher the proportion of the copolymer, the lower the LCST.³¹ Here, copolymerizing PNIPAAm film with polydopamine reduced the LCST from 32 to 30.4°C. The drop of LCST was not significant because the proportion of the polydopamine in the film is relatively small compared to PNIPAAm. Nevertheless, the enthalpy of transition confirmed the deposition of PNIPAAm onto polydopamine film. Moreover, the phase transition behavior of the PNIPAAm-*g*-polydopamine polymer film dissolved in 0.1 N NaOH was monitored by measuring the transmission of visible light at 563 nm, as a function of temperature. As shown in Figure 5B, the cloud point temperature was determined to be 30.5°C which is in line with the LCST detected by DSC thermogram.

In order to characterize the hydrophilicity/hydrophobicity change of the PNIPAAm-*g*-polydopamine surface, water contact angles of bare, polydopamine coated, and PNIPAAm-*g*-polydopamine coated Petri dishes were measured at ambient temperature and 40°C (Figure 6). The water contact angle of bare Petri dish at ambient temperature was 70.3° ± 1.8°. It slightly increased to 71.7° ± 0.5° when it was heated up to 40°C. The water contact angle of the polydopamine coated Petri dish was 48.9° ± 2.9° at ambient temperature and 49.8° ± 3.0° at 40°C. The polydopamine coating makes the Petri dish more hydrophilic, which was consistent with the findings of Liu et al.³³ The water contact angle of the produced PNIPAAm-*g*-polydopamine coating at the ambient temperature was 36.1° ± 2.7°, indicating the produced PNIPAAm film was even more hydrophilic than the polydopamine film at ambient temperature. When heated up to 40°C, the contact angle on PNIPAAm-*g*-polydopamine coated Petri dish increased to 82.1° ± 4.2°. The results indicated that when the bare and polydopamine coated Petri dishes were heated up, the surfaces did not exhibit temperature-responsive hydrophobicity according to the measured contact angles. However, the dramatic increase of water contact angle on PNIPAAm-*g*-polydopamine indicated that the surface switched from hydrophilic to hydrophobic when it was heated up over its LCST.

3.4. Culture and Harvest of hMSCs on PNIPAAm-*g*-polydopamine surface

In order to characterize the cell detachment behavior of PNIPAAm-*g*-polydopamine surface, hMSCs were cultured onto bare, polydopamine coated, and PNIPAAm-*g*-polydopamine coated Petri dish. For detachment studies, the environment temperature of hMSCs was reduced from 37°C to ambient temperature by replacing the culture medium with 1 mL fresh

α MEM. The hMSCs grown on all three surfaces were given a mechanical disruption by a gentle pipetting force at 20, 40, and 60 min.

Our results showed that at temperature of 37°C (above the LCST), hMSCs were able to attach to all three surfaces. After the treatment of cells with ambient temperature α MEM for 1 h, no significant cell detachment was observed on either bare or polydopamine controls (Figure 7A and 7B). A very small amount of cell detachment was observed from bare or polydopamine coated Petri dishes (Figure 8) 1 h after the temperature switch. As shown in Figure 8, only $3.1 \pm 0.2\%$ of the cells detached from bare Petri dish, and $4.3 \pm 0.3\%$ of the cells detached from the polydopamine coated Petri dish. On the other hand, the PNIPAAm film became expanded and hydrated, and hMSCs started to detach from the surface. The hMSCs grown on PNIPAAm-*g*-polydopamine surface showed changes in shape, from a stretched, spindle shape toward a circular shape over the span of 60 min (Figure 7C). Within 40 min, an average of $44.8 \pm 3.5\%$ of the cells detached from the PNIPAAm-*g*-polydopamine surface after the temperature switch. At 60 min, $78.4 \pm 5.2\%$ of the cells grown on the PNIPAAm-*g*-polydopamine Petri dish detached from the surface (Figure 8). It is noteworthy that cells having smaller size (i.e., human epithelial lung carcinoma A549 cells) rounded up faster than hMSCs. According to our results (Figure S1), A549 cells started to round up at 20 min, and 90% of the A549 cells detached from the PNIPAAm-*g*-polydopamine surface at 40 min. The percentage of detached hMSCs was not 100% probably because the PNIPAAm film was not uniformly deposited on the Petri dish surface (Figure 2A). In certain areas, the amount of deposited polymer was probably not enough to lift up all of the hMSCs. This issue could be improved by the increment of PNIPAAm deposition with higher concentration of dopamine.

The time for cells to detach from PNIPAAm and its copolymer surfaces typically ranges from 2 min to 2 h.³⁴ Cell type has a remarkable effect on the detachment time from a PNIPAAm surface.^{35–37} Our results showed that hMSCs needed up to 60 min to detach from the PNIPAAm-*g*-polydopamine surface probably because hMSCs need a larger force to detach themselves than cells smaller in size such as A549 and NIH 3T3 cells. The 30 min detachment time of A549 cells from PNIPAAm-*g*-polydopamine surface was comparable to the reported 30 min detachment time of retinal pigment epithelial cells from UV polymerized PNIPAAm surface.³⁸ From our results, we have demonstrated that PNIPAAm was successfully synthesized on the surface of Petri dish through polydopamine coating. Human MSCs were able to attach and proliferate onto PNIPAAm-*g*-polydopamine surface, and the cells can be harvested by reducing the temperature below LCST.

The detached hMSCs from PNIPAAm-*g*-polydopamine coated Petri dishes were further inoculated on freshly prepared PNIPAAm-*g*-polydopamine coated dishes. The re-plated hMSCs were able to attach, spread, and proliferate on PNIPAAm-*g*-polydopamine coated dishes. Figure 9 shows their growth kinetics. The plating density of 10^3 cells/cm² revealed 51-fold expansion after 12 days. It should be noted that over the course of culturing A549 cells using DMEM medium, the polydopamine film would collapse if the pH of the culture medium turned acidic after several days of culturing (Figure 10). This is probably because the pH change due to the production of lactic acid caused the deformation of the polydopamine film. Most cell growth occurs at an environment of neutral pH. The pH drops when lactic acid is produced as the by-products of cellular metabolism. According to our

findings, the acidic environment led to the deformation of the polydopamine film, which is consistent with the reported finding.³⁹ The deformed PNIPAAm-*g*-polydopamine film would be harvested along with the detached cells as unwanted by-products. Therefore, maintaining the pH of culture medium at a neutral level is of importance for cell harvest from the temperature-responsive surface reported here.

4. Conclusions

In this study, free radical polymerization is combined with polydopamine chemistry to develop a straightforward and cost-effective way of preparing PNIPAAm surface for temperature-modulated cell harvesting. AFM images showed a layer of PNIPAAm film was grafted onto the allyl functionalized polydopamine coated substrata, with randomly distributed PNIPAAm polymer aggregates on the substrate surface. FTIR and NMR confirmed the formation of PNIPAAm-*g*-polydopamine polymer film on the surface. Water contact angle and DSC thermogram revealed the LCST of the PNIPAAm-*g*-polydopamine film at 30.4°C. After the environmental temperature changed from 37°C to ambient within 60 min, over 80% of cultured hMSCs detached from the PNIPAAm-*g*-polydopamine surface. The detached hMSCs after re-plating were able to spread and proliferate on newly prepared PNIPAAm-*g*-polydopamine coated dishes. In summary, polydopamine has provided a versatile platform for PNIPAAm grafting, and the fabricated PNIPAAm-*g*-polydopamine film can be used as temperature-responsive surface for cultivation and harvest of human mesenchymal stem cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Reaction scheme for grafting PNIPAAm onto allyl functionalized polydopamine.

Figure 2. AFM analysis of (A) allyl-polydopamine coated and (B) PNIPAAm-*g*-polydopamine coated Petri dish. The experimental results were presented as (a) topographic image, (b) spectrum of sectional heights, and (c) 3D image.

Figure 3.
FTIR spectra of freeze-dried PNIPAAm, polydopamine, and PNIPAAm-*g*-polydopamine.

Figure 4.
NMR spectrum of PNIPAAm-*g*-polydopamine.

Figure 5. (A) DSC thermogram of PNIPAAm-*g*-polydopamine dissolved in 0.1 N NaOH. (B) UV-Vis transmittance at 563 nm of PNIPAAm-*g*-polydopamine film dissolved in 0.1 N NaOH as a function of temperature. The cloud point temperature (i.e., 30.5°C) corresponds to the transmittance which is 50% of the initial value.

Figure 6.

Water contact angles of (A) bare, (B) polydopamine coated, and (C) PNIPAAm-g-polydopamine coated Petri dish at ambient temperature and 40°C. Water contact angles were expressed as Mean $\pm$ S.D. (n=3). $p < 0.05$ is considered to be statistically significant.

Figure 7. Phase contrast microscopic images of hMSCs on (A) bare, (B) polydopamine coated, and (C) PNIPAAm-*g*-polydopamine coated Petri dish for 1 day. Then, the cells were taken out into ambient temperature, and phase contrast images were taken at 0, 20, 40, and 60 min, respectively. Scale bar denotes 100 μ m.

Figure 8.

Percentage of hMSCs detached from bare Petri dish (◆), polydopamine coated Petri dish (■), and PNIPAAm-*g*-polydopamine coated Petri dish (▲) due to the temperature switch. The numbers of detached cells were counted at 20, 40, and 60 min, respectively. Error bars indicate S.D. (n=3). $p < 0.05$ is considered to be statistically significant.

Figure 9.

(A) Growth kinetics of hMSCs detached from PNIPAAm-*g*-polydopamine coated Petri dishes and re-plated on new PNIPAAm-*g*-polydopamine coated Petri dishes. Inoculation density was 10³ cells/cm². Error bars indicate S.D. (n=3). $p < 0.05$ is considered to be statistically significant. (B) Phase contrast images of hMSCs re-seeded on new PNIPAAm-*g*-polydopamine coated Petri dish at day 0, 3, 6, 9, and 12. Scale bar denotes 100 μm.

Figure 10.

A549 cells were cultured on PNIPAAm-*g*-polydopamine coated Petri dish. After several days of culturing, the color of DMEM culture medium turned yellow, and PNIPAAm-*g*-polydopamine film collapsed. Scale bar denotes 100 μm .