Noninvasive Photodetachment of Stem Cells on Tunable Conductive Polymer Nano Thin Films: Selective Harvesting and Preserved Differentiation Capacity

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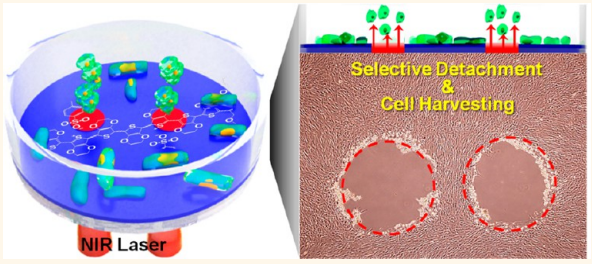
ABSTRACT Viable mesenchymal stem cells (MSCs) were efficiently and selectively harvested by near-infrared (NIR) light using the photothermal effect of a conductive polymer nano thin film. The poly(3,4-ethylenedioxy thiophene) (PEDOT)-coated cell culture surfaces were prepared *via* a simple and fast solution-casting polymerization (SCP) technique. The absorption of PEDOT thin films in the NIR region was effectively triggered cell harvesting upon exposure to an NIR source. By controlling the NIR absorption of the PEDOT film through electrochemical doping or growing PEDOT with different thin film thickness from 70 to 300 nm, the proliferation and harvesting of MSCs on the PEDOT surface were controlled quantitatively. This light-induced cell detachment method based on PEDOT films provides the temporal and spatial control of cell harvesting, as well as cell patterning. The harvested stem cells were found to be alive and well proliferated despite the use of temperature increase by NIR. More importantly, the harvested MSCs by this method preserved their intrinsic characteristics as well as multilineage differentiation capacities. This PEDOT surfaces could be used for repetitive culture and detachment of MSCs or for efficient selection or depletion of a specific subset from heterogeneous population during culture of various tissue-derived cells because there were no photodegradation and photobreakage in the PEDOT films by NIR exposure.



KEYWORDS: cell harvesting · conductive polymer thin film · photothermal effect · cell engineering · stem cell

The target cell detachment at a defined region is of great interest in the biomedicine researches including cell biology, cell based analysis for drug development, regenerative medicine.[1−4] This target cell harvesting has been performed using stimuli, for example, electro-, thermo-, and photochemical stimuli, responsive underlying cell culture surfaces.[5−9] Among external stimuli, light-induced detachment offers the ability to accomplish spatial control of cell detachment and is less harmful than conventional enzyme treatment techniques that may cause severe damage to the cell membrane and protein adhesion. Argitis *et al.* and Revzin *et al.* reported ultraviolet (UV) light-induced cell detachment with photodegradable polymer substrates and photolabile linkers, respectively.[10,11] However, these UV light approaches have intrinsic shortcomings that limit their utilization in *in vivo* applications: UV light is only suitable for *in vitro* studies due to its quick attenuation in soft tissue and UV light may cause severe damage to tissue and biomolecules. Furthermore, the previous approaches require dedicated efforts and time-consuming processes, such as either sophisticated synthesis or an extra surface modification for UV responsive cell culture plates. Importantly, most previous studies regarding light induced cell detachment utilized irreversible photoreaction by using photodegradable polymer or photolabile linkage, which resulted in no reusable cell culture plates after first light irradiation. Recently, Sada *et al.* reported NIR light-induced cell detachment

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with carbon nanotube (CNT)-coated substrates, which were capable of converting NIR absorption to photothermal and phtoacoustic effects.[3] Depite the use of NIR for cell detachment, there are still several disadvantages such as non easy process arising from CNT dispersion problem, the requirement of high energy resulted from the use of pulse laser, and broader light absorption of CNT from UV−vis to NIR.

In this paper, we are the first to report efficient cell harvesting and spatially controlled stem cell detachment using the NIR-induced photothermal effeact of PEDOT thin films with easily tunable photophysical properties. Therefore, we believe that the advantages of our studies over previous studies using UV−vis source and CNT layer are to use biofriendly light source and organic polymer films. Compared to UV−vis, near-infrared (NIR) light does not interfere with tissue and is highly transmissive to tissue without damage. Also, the advantage of organic polymer films (PEDOT) over CNT layer is easy to tune their photophysical properties including photothermal effects with doping level as well as chemical modification. Conductive polymers (CPs) have been extensively examined for electro- or photon-mode control of organic devices due to their easy control of the band gap energy in the visible to near IR regions.[12−15] Furthermore, CPs are of considerable interest to various biomedical applications, such as the control of neural stem cells in tissue engineering, neural probes as implantable electrodes, and biosensors, because their electroactive properties change the conductivity, color, morphology, and volume in response to external stimuli.[5−7,16−20] CPs absorb NIR light depending on their doping states.[12,13,20] Through nonradiative energy dissipation and exothermic photochemical reactions,[21] such absorption of the NIR light by a CP surface could generate photothermal effect, which should be useful for cell harvesting. Furthermore chemical change in CP of the culture surface could allow further control of cell detachment. Therefore, we performed a noninvasive photodetachment of stem cells using cell culture surfaces coated with an NIR sensitive CP, poly(3,4-ethylenedioxy thiophene) (PEDOT). PEDOTs have been applied in various bioengineering applications, such as bioactuators, drug-releasing vehicles, and scaffold, due to their structural stability, high aqueous stability, and biocompatibility.[5,6] The PEDOT-coated cell culture surfaces were prepared *via* simple solution-casting polymerization (SCP), which allows us to easily control the film thickness as well as to coat the functional polymer film on a large area substrate.[22] Then the chemical change in CP of the culture surface was carefully varied by electrochemical doping.

RESULTS AND DISCUSSION

Preparation, Characterization, and Biocompatibility of PEDOT Thin Films. PEDOT coated cell culture surface and harvesting by NIR are schematically shown in Figure 1. PEDOT films with thicknesses of 70−300 nm were easily and directly coated to polystyrene Petri dishes or ITO glass through SCP. Pristine PEDOT films (SP) were *p*-doped with tosylate and were transparent blue (Figure 1a,b). Partially doped PEDOT (P1P and P2P) and dedoped PEDOT (neutral PEDOT, NP) film-coated surfaces were prepared by electrochemical dedoping of the SP surface *via* a cyclic voltammetry by stopping the potential at +0.4, −0.2, and −1 V, respectively (Figures 1a,b and 2a). The color of the NP was deep purple blue (Figure 2b), which is characteristic of the dedoped state. The partially doped PEDOT films (P1P, P2P) clearly showed intermediate color between SP and NP films (Figure 2b). Film adhesion is also

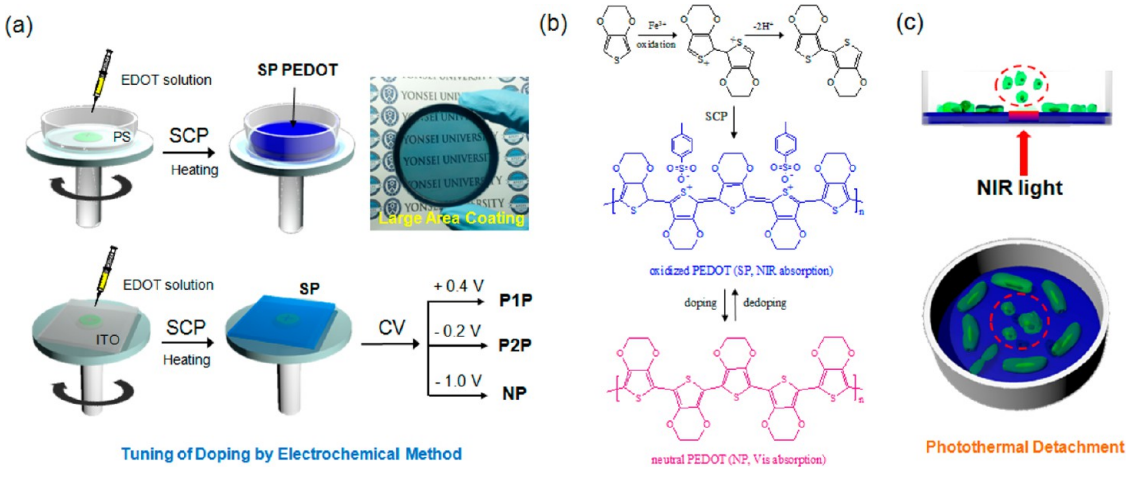

Figure 1. Synthesis and fabrication of conductive polymer (PEDOT) thin films and tailor of their doping levels *via* electrochemistry for NIR induced cell detachment. Schematic illustration of (a) the preparation of PEDOT films on a Petri dish or ITO glass *via* SCP and then the control of doping level of pristine SP film on ITO *via* electrochemistry. (Inset: photographic image of SP film coated on Petri dish.) (b) Synthesis of heterocyclic conductive polymer (PEDOT) by oxidative polymerization and the chemical structures of PEDOT at doped and dedoped states. (c) Schematic diagram of NIR-induced MSC detachment from a PEDOT film.

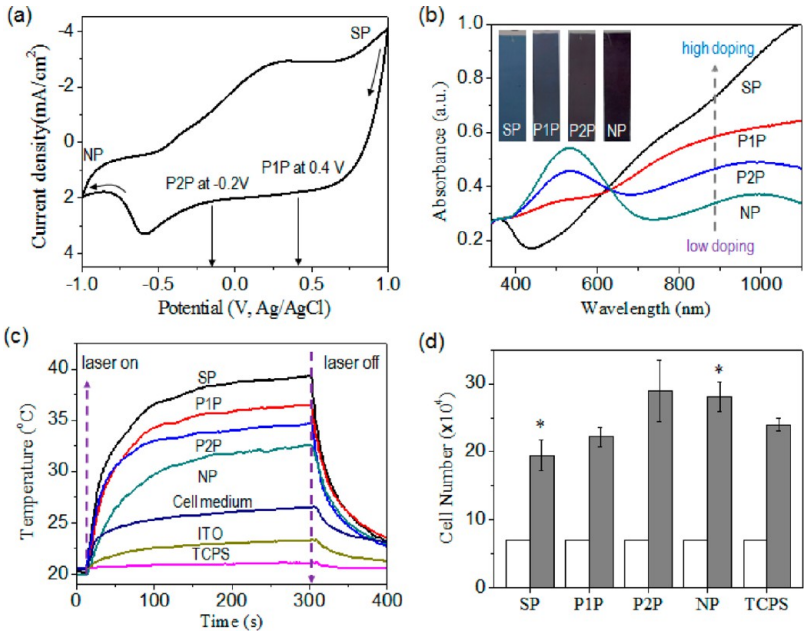

Figure 2. Control of doping level of pristine PEDOT film *via* electrochemistry and the effects of PEDOT doping level on NIR absorption, temperature increase, and MSCs proliferation. (a) Cyclic voltammogram of PEDOT coated on an ITO glass electrode in 0.1 M $LiClO_4$ solution *vs* Ag/AgCl (stainless steel as counter electrode; second cycle). The cyclic voltammogram was obtained at a 50 mV s^{-1}. (b) The absorption spectra of SP, P1P, P2P, and NP. Inset: photographic images of SP, P1P, P2P, and NP coated to ITO. (c) Photothermal effect of the Petri dish, ITO, and PEDOT films upon NIR irradiation (808 nm, 230 mW, 69 J). The NIR light was turned off after 5 min. (d) Proliferation rate of MSCs cultured on PEDOT films and TCPS for 3 days (black bar) from day 0 (white bar). MSCs were placed at the number of 63 000 cells at first (white bar); *$p < 0.05$ compared to TCPS.

important for the successful application of a conductive polymer to bioengineering. Both PEDOT films coated on the Petri dish and ITO glass exhibited good adhesion, which was evaluated using a standard tape test. The PEDOT films on the substrate did not show any indication of damage or peeling from the substrate over a long period of cell cultivation and experiments of 14 days. As shown in the absorption spectra presented in Figure 2b, the NIR absorption of SP films was strong due to the formation of polaronic and bipolaronic structures. Additionally, the NIR absorption of PEDOT films was strongly dependent on the doping level. NIR absorption gradually decreased as PEDOT films changed to the dedoped state. The absorption of the SP film at 808 nm was 2.2 times greater than that of the neutral PEDOT film (NP).

The photothermal effect of PEDOT surfaces by NIR absorption was determined from the temperature change of the PEDOT surfaces by NIR irradiation (808 nm, 230 mW) for 5 min (Figure 2c). NIR irradiation of the SP surface in cell media (DMEM, 3 mL) led to a much greater temperature increase of SP surface than those of TCPS (0.7 °C) and ITO (2.9 °C). The temperature of the liquid cell media (DMEM), 2 mm at the height apart from the SP surface, exhibited a temperature increase (5.9 °C) likely due to heat transfer from the SP surface to the cell media during NIR exposure. The temperature increase of PEDOT films triggered by NIR exposure was strongly dependent on the doping level of PEDOT films. The temperature rise decreased as the doping level of PEDOT film decreased, which corroborates with the NIR absorption change of PEDOT films, as shown in Figure 2b. Thermal images recorded with an IR camera clearly showed the temperature rise due to photothermal effect (Figure S2). Contrast to the standard tissue-culture polystyrene (TCPS) dish, PEDOT (SP) films exhibited a distinct color change from blue to deep red, upon NIR exposure. Additionally, lateral thermal diffusion was characterized with PEDOT (SP) films of different thickness. As shown in Figure S3, the thicker film showed higher maximum temperature. However, considering the slope of the temperature decrease from NIR exposed region to nonexposed (outside) region, thermal diffusion property seems to be almost similar, independent of the film thickness. In contrast to metal film layers such as gold and aluminum,[23] organic PEDOT films exhibited almost the same lateral thermal diffusion for the films of different thicknesses. This could be attributed to the low thermal conductivity of PEDOT films. It has been reported that the thermal conductivity (κ) is 0.37 W m^{-1} K^{-1} for poly(3,4-ethylenedioxy thiophene) (PEDOT).[24] Therefore, in the PEDOT thin films, the thickness of the films affected temperature increase mainly on the light exposed confined region. To investigate the proliferation rate of mesenchymal stem cell (MSC), cells were cultured on five plates: TCPS, SP, P1P, P2P, and NP. Figures 2d and S4 show the proliferation rate and microscope images of MSCs cultured for 3 days, respectively. MSCs cultured on SP surface increased from 6.3×10^4 to 2.0×10^5 (3.2-fold) at

day 3. MSCs on SP surface showed a slightly lower proliferation rate compared to those on TCPS (3.7-fold). The cell morphology on the SP surface showed partial MSCs aggregation (Figure S4). Nonetheless, it was believed that the SP surface could be used for a cell culture substrate. MSCs cultured on NP film increased from 6.3×10^4 to 2.8×10^5 (4-fold) at day 3. The proliferation rate of MSCs cultured on NP film was larger than SP, and even greater than TCPS. MSCs cultured on NP had normal cell morphology, were well-distributed, and highly proliferated on the entire surface. Interestingly, MSCs on a partially doped surface (P1P) exhibited cell morphology similar to that on TCPS and on NP surface, and furthermore, they showed a higher proliferation rate than the SP surface. FE-SEM images of SP and NP, shown in Figure S5a,c, indicated no significant difference in morphology between the SP and NP surface at the microscale. Thus, the higher proliferation rate on NP and P1P could be attributed to the lower doping density at the PEDOT surface.

The biocompatibility of the PEDOT films was determined from a cell apoptosis experiment using MSCs and quantified using 7-AAD (Figure S6).[25−28] The cell viability on TCPS, SP, and NP was 99.91, 98.25, and 99.11%, respectively, indicating high biocompatibility of the surfaces coated with PEDOT both at dedoped (neutral) and doped states.

NIR Triggered Cell Harvesting. The effect of NIR light on cell viability was also examined in the absence of PEDOT films. Thus, a propidium iodide (PI) staining experiment was performed after NIR exposure to MSCs cultured on the TCPS surface without PEDOT films. After NIR exposure (808 nm, 230 mW) for 30 min, MSCs were firmly adhered to TCPS in the NIR-exposed area, with no noticeable change in their morphology (Figure S7a). The fluorescent microscope image in the NIR exposed area did not show any red fluorescence (Figure S7b), indicating that MSCs were alive. It is well-known that PI staining become positive with vivid red fluorescence in dead cells due to loss of membrane integrity and free intercalation with the double stranded DNA in dead cells. Importantly, MSCs were well proliferated in a confluent state. Therefore, the NIR irradiation did not cause any damage to MSCs under these conditions. The temporal and spatial control of MSC detachment upon NIR exposure was performed by three consecutive steps: (1) PEDOT film coated surfaces were prepared *via* SCP and tuned doping level *via* electrochemistry, (2) MSCs were cultured on the PEDOT films coated surfaces for 2−3 days, and (3) NIR light (808 nm, 230 mW) was exposed to cells cultured on PEDOT surfaces for MSC detachment, patterning, and harvesting (Figures 1 and S1). Figure 3a shows that cell detachment was strongly related to

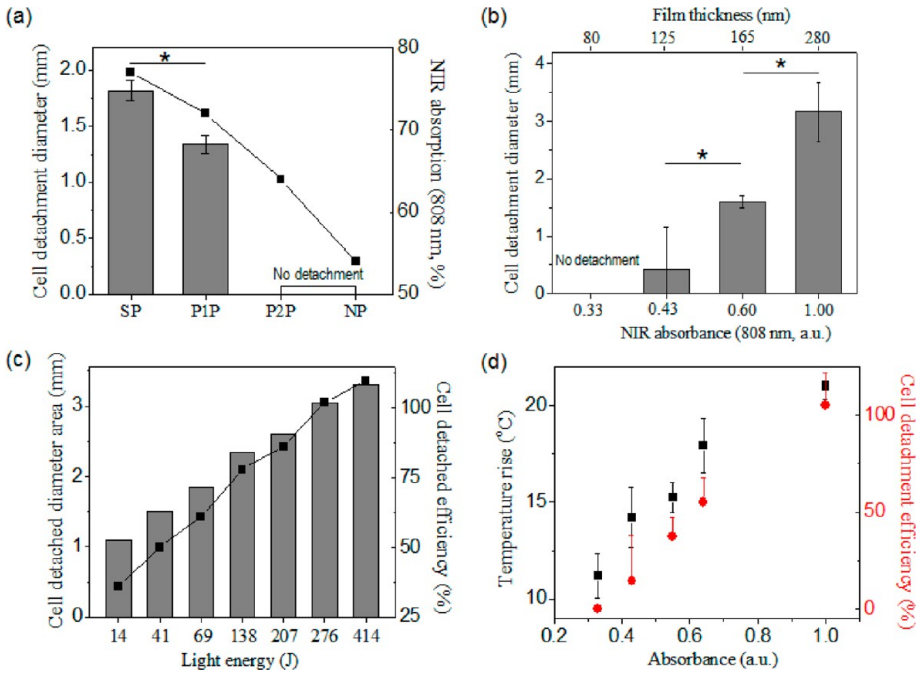

Figure 3. Cell detachment area and efficiency dependent on PEDOT doping level, thickness, and NIR light energy. (a) Diameter of the detached area of MSCs (bar) on PEDOT films (SP, P1P, P2P, and NP) upon NIR exposure (808 nm, 230 mW, 207 J) for 15 min and absorbance at 808 nm of the PEDOT surfaces (square); *$p < 0.05$. (b) Average diameter of the detached area of MSCs (bar; three experiments) on SP films with different thicknesses (280, 165, 125, and 80 nm) upon NIR exposure (808 nm, 230 mW, 207 J) for 15 min; *$p < 0.05$. (c) Diameter of the detached area of MSCs (bar) and efficiency of cell detachment (square) on the SP film (thickness = 280 nm) surface depending on the NIR (808 nm, 230 mW, 14 to 414 J; beam diameter size: 3 mm) light energy. (d) Correlation of temperature increase (square) and cell detachment efficiency (circle) with NIR absorbance (808 nm) of PEDOT films.

TABLE 1. NIR Absorption and Cell Detachment Diameter As a Function of the PEDOT Doping Level and Film Thickness

	SP	P1P	P2P	NP	SP			
thickness (nm)	174	[a]	[a]	[a]	80	125	165	280
A_{808}[b] (a.u.)	0.68	0.52	0.42	0.29	0.33	0.43	0.60	1.00
D[c] (mm)	1.82 ± 0.09	1.34 ± 0.08	n^d	n^d	n^d	0.42 ± 0.73	1.60 ± 0.10	3.16 ± 0.52

[a] Partially doped PEDOT (P1P and P2P) and dedoped PEDOT (NP) films were prepared by electrochemical dedoping of pristine PEDOT films (SP, thickness = 174 nm) *via* a cyclic voltammetry. [b] NIR absorbance of PEDOT films at 808 nm. [c] Diameter of the detached area of MSCs on PEDOT films upon NIR exposure (808 nm, 230 mW, 207 J) for 15 min. [d] Cell detachment was not observed.

the doping state of PEDOT films, likely due to the different temperatures from different NIR absorption of PEDOT films. In contrast to P2P and NP films, the highly doped PEDOT films, such as SP and P1P, which were expected to generate more heat, clearly exhibited cell detachment as they have high absorbance (A) in the NIR region ($A_{808} \geq 0.55$). The cell detachment on PEDOT films triggered by NIR exposure was also dependent on the SP film thickness. As shown in Figure S8, the thicker SP films (280 and 165 nm) revealed much greater NIR absorption and an increase in temperature than the thinner SP films (125 and 80 nm). As a result, more cells were detached from the thicker SP films that absorbed more NIR light ($A_{808} \geq 0.6$; Figure 3b). Surprisingly, MSCs cultured on thicker SP film (280 nm) were detached very fast. The cell detached diameter was larger than 1 mm, yielding a detachment efficiency (ξ), the ratio of the detached area to NIR exposed area, of ~40% within 1 min (Figure 3c). Additionally, we have characterized the spatial resolution of our cell detachment system by changing NIR beam size with iris diaphragm at 100% cell detachment efficiency condition. As shown in Figure S10, the cell detached area was nearly the same as the irradiated beam size, indicating the cell detachment on conductive polymer can be controlled precisely by NIR beam size and moreover, importantly, shows the possibility of application to single cell detachment. As a result, the area of detached MSCs and ξ were dependent on the NIR light energy and area, which could be useful for controlled detachment. Therefore, the cell detachment could be further optimized by varying the objective lenses and using a light expander to meet the requirement. As shown in Figure 3d, the temperature increase of all samples was well correlated to the NIR absorbance (A_{808}) of the surface, which was well matched to the efficiency of cell detachment. Table 1 summarized the NIR absorption and cell detachment diameter as a function of the PEDOT doping level and PEDOT film thickness. This result verifies that the cell detachment is based on the photothermal effect of PEDOT. Moreover, it provides a reliable clue for assessing the efficiency of cell detachment by simply determining the color or NIR absorbance (A_{808}) of the surface.

Figure 4a,b clearly shows the selective detachment of MSCs when NIR light was irradiated on MSCs cultured on PEDOT films (SP and P1P). There was no change or impact on the PEDOT surface morphology after NIR exposure (230 mW, 414 J) for 30 min, as shown by FE-SEM images of PEDOT(SP) before and after NIR irradiation (Figure S5a,b), indicating that the NIR irradiation for MSC detachment did not result in a change of the PEDOT surface morphology or damage to the surface. The cell release might be explained by the temperature rise on the polymer surface based on the conversion of NIR absorption into heat generation on the binding of integrin and ECM proteins. It was reported that the contact area of cells adhered to a surface *via* integrins is quite small and only a few percent of the surface coverage.[28] Therefore, one of the major reasons for the cell release could be ascribed to the temperature increase in the PEDOT surface due to NIR exposure, which leads to a few bond breaks between the integrin and extracellular matrix (ECM). Because integrin reorganization is dynamic in response to microenvironmental change,[29] such as temperature, the reason for cell detachment might be attributed to the loss of the linkage between integrin and ECM proteins by heat generated from the photothermal effect of PEDOT.

Furthermore, FT-IR spectrum of the PEDOT, recovered from the NIR exposure of PEDOT film in cell culture media, showed no change when compared with that of the fresh unexposed PEDOT film in the entire spectral range (Figure S9a). The conductive polymer C=C peak at 1540−1480 cm^{-1} and ECM protein peaks at N−H and C=O stretching peak at 1450 and 1600 cm^{-1} were all intact for the recovered PEDOT film from the NIR experiments.[22] Moreover, the PEDOT films were stable for repetitive NIR exposure (>10 times) as confirmed from cyclic voltammograms for NIR exposed films (Figure S9b). These results confirmed that the PEDOT surface and ECM proteins absorbed on PEDOT surface are stable and maintain their properties in the cell harvesting by NIR. Though it was reported that mammalian fibroblasts cultured on cell media of high temperature (43−45 °C) for 1 h showed reduced viability,[30,31] it is important to note that the NIR induced temperature increase in our system was localized only on the NIR exposed area of the PEDOT surfaces and did not cause the significant temperature increase in cell media (Figure 2c).

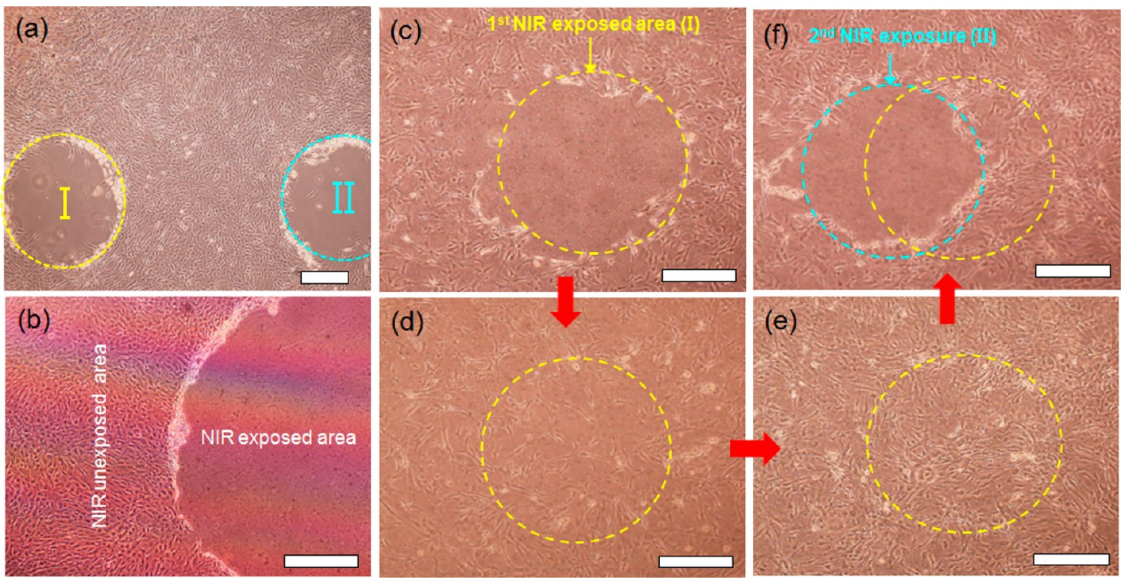

Figure 4. Selective cell detachment on desired cell culture region and consecutive cell detachment on the same area. Optical microscope image of MSC detachment (a) on SP film upon NIR exposure (808 nm, 230 mW, 207 J) for 15 min and (b) on PEDOT (P1P) upon NIR exposure (808 nm, 230 mW, 276 J) for 20 min (scale bar = 500 μm). Consecutive optical microscope images of the reusable PEDOT surface for MSC detachment. (c) MSC detachment (yellow circle) on P1P PEDOT upon first NIR exposure (808 nm, 230 mW, 207 J) for 15 min. (d, e) MSC spreading on the previously detached area (yellow circle) after 1 and 2 days. (f) Repeated MSC detachment (sky blue circle) upon NIR exposure (808 nm, 230 mW, 207 J) for 15 min. Scale bar = 500 μm.

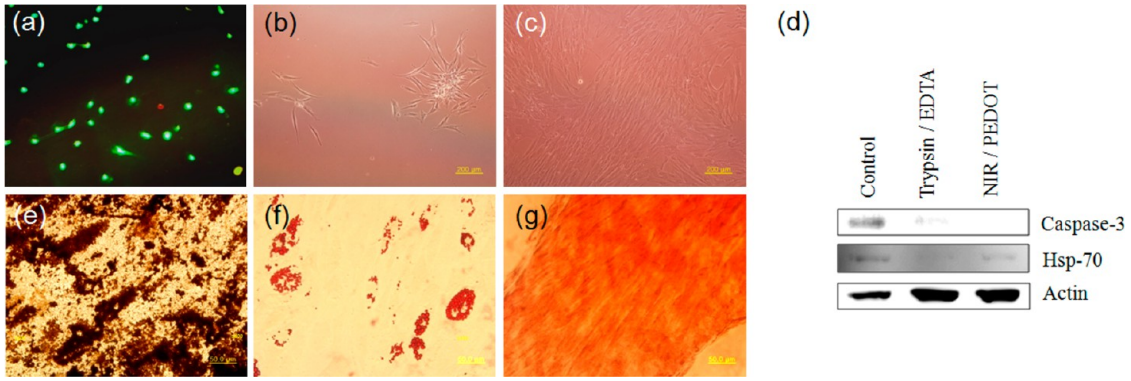

Figure 5. Verification of the viability and differentiation capacity of MSCs detached by NIR exposure. MSCs were detached from the SP and P1P surfaces by NIR exposure (808 nm, 230 mW, 207 and 276 J) for 15 and 20 min to analyze cell viability and differentiation capacity, respectively. (a) The viability of MSCs detached by NIR exposure as demonstrated by Live-dead staining. Optical microscope images for the recovered MSCs cultured on TCPS (b) after 1 day and (c) 7 days. (d) Caspase-3 and Hsp-70 on apoptosis-associated cell damages, examined by Western blot. Dead cells were used as a control. Cells were detached by trypsin/EDTA and by NIR laser from PEDOT substrate, respectively. (e–g) Images for differentiation assay of the recovered MSCs into (e) osteogenesis, (f) adiopogenesis, and (g) chondrogenesis.

The effects of localized temperature increase on MSCs viability and MSCs functions were more explained below with Figures 5 and S12.

Selective MSC Harvesting and Reusability of the PEDOT Surface for Repetitive Culture and Detachment of MSCs. Figure 4a shows the temporal and spatial control of MSC detachment using PEDOT films in two different regions. After the first 15 min of NIR exposure (230 mW, 207 J) to MSCs cultured on SP surface, MSCs were detached to expose a bare circular region with a 1.5 mm diameter (region I). In the next step, the NIR light was moved and region II with attached cells was exposed for 15 min and cells were harvested, revealing another bare circle pattern on the surface (region II). Thus, multiple NIR exposures for cell detachment were possible, resulting in selective detachment of desired areas and cell patterns on the cell culture surface. Immediately after NIR exposure and before pipetting for detachment, it was observed that the morphology of the MSCs on the NIR exposed region was different from that on the unexposed region (Figure S11). MSCs on the NIR exposed region were unstable and detached over a long period (5–6 h) even without pipetting. Additionally, MSCs were clearly detached immediately after NIR exposure using moderate mechanical stimulations, either repetitive pipetting or shaking of the cell culture chamber. PEDOT films have

the significant advantage over thermoresponsive polymers such as poly(*N*-isopropylacrylamide)s (PNiPAAm), which are widely used for cell detachment upon a change in temperature.[32] Contrast to PNiPAAm, PEDOT films allow us to accomplish the spatial control of cell detachment which could make the PEDOT film as selective cell harvesting and cell patterning surface. The PEDOT surfaces after one cycle of cell culture and detachment could be reused for repetitive culture and detachment of MSCs in the same region because there were no surface defects or morphology changes after NIR exposure. After the first selective detachment and harvest of MSCs from the NIR-exposed area (Figure 4c), the PEDOT surface was maintained in cell culture media for 2 days. As shown in Figure 4d,e, the entire area of the surface was covered with MSCs without the bare circular area, indicating that the PEDOT surface after the first harvest was reusable as a culture surface and the cell culture was extended to the NIR-exposed circular area. When NIR light was focused for 15 min onto the first harvest area and adjacent area of the surface (sky blue circle in Figure 4f), MSCs were completely detached from the exposed area, including the first harvested area, to reveal a 1.3 mm diameter bare circle. Such reusable surface of PEDOT films, showing temporal and spatial control of cell detachment, never been reported and this is the first example of a cell harvesting system by NIR exposure.

Viability, Attachment, Proliferation, and Differentiation Capacity of NIR-Harvested MSCs. The viability of the harvested MSCs by NIR (230 mW, 207 and 276 J) was examined first by using a Live/Dead viability/cytotoxicity kit. As shown in Figure 5a, the viability of MSCs detached by NIR exposure exceeded 95%. In addition, the harvested MSCs from NIR-triggered detachment were seeded onto a TCPS surface in fresh cell media. As shown in Figure 5b,c, the harvested MSCs were alive and stably attached to the TCPS. Moreover these MSCs proliferate normally to confluence after 7 days. To further investigate the heat effect on cells, we carried out Western blot analysis to identify the expression of specific proteins related on apoptosis-associated cell damages. As shown in Figure 5d, Caspase-3, which is expressed in cells during apoptosis as an aspartate specific cysteine protease,[33] was not observed in MSCs detached from SP by NIR exposure. Compared to the dead cell control, heat shock protein-70 (HSP-70), which is expressed in response to heat stress and stressful stimuli and then operate the protection system against cell apoptosis,[34] was a little observed in MSCs detached by NIR, which might be induced by the heat generated from NIR exposure. Taken together, the temperature increase on the SP substrate, triggered by NIR exposure, affects MSCs as stressful stimulus, however, it is obvious that MSCs detached by this method remained alive. This NIR method was comparable to the cell detachments with trypsin treatment.

Human MSCs are multipotent and have been recognized as a source for tissue engineering or cell therapy for a broad spectrum of human diseases that clearly require the extensive expansion of MSCs *in vitro* while preserving their multipotency. Therefore, the preservation of cellular integrity of the NIR-detached MSCs is critical for practical application of the NIR method to cell engineering and extensive study of harvested stem cells in biomedical engineering. Immunophenotyping using negative marker (CD34) and positive markers (CD29 and CD90) revealed also that harvested MSCs by NIR exposure maintained their intrinsic properties without any change of surface proteins (Figure S12).[35] These results strongly support that the NIR-harvested MSCs preserve cellular integrity and encourage us to carry further experiments such as differentiation. To investigate the differentiation capacity of the harvested cells, the harvested MSCs were separately differentiated for osteogenesis, adipogenesis, and chondrogesis in specific differentiation media for three weeks. The harvested MSCs were successfully differentiated into osteogenic, adipogenic, and chondrogenic lineages, indicating that they preserved their multilineage differentiation capacities (Figure 5e–g). Overall, the analysis of live and dead staining, normal cell proliferation, Western blot analysis, immunophenotyping, and well-preserved differentiation capacity of the harvesting cells strongly indicate that NIR induced cell harvesting is not invasive and noncytotoxic despite of the use of temperature increase by NIR.

CONCLUSIONS

In conclusion, the stem cell harvesting using a CP-coated surface in this work is triggered by NIR light that generate heat on the light exposed area of the surface, leading spatially controllable cell detachment unlike typical thermal detachment methods. The photothermal effect of PEDOT films by NIR light plays an important role in the cell harvesting, the selective cell detachment, and patterning of viable cells. As the photothermal effect increased by the degree of the doping in PEDOT, the harvesting of MSCs by NIR exposure was most effective in a highly doped PEDOT (SP). However, proliferation of MSCs was faster in a less doped PEDOT. Thus, proliferation and harvesting could be optimized simply tuning the degree of doping in PEDOT electrochemically and, thus, a partially doped PEDOT (P1P) film showed fast proliferation with normal cell morphology plus effective cell harvesting. Other factors for harvesting were light energy and exposure area. By increasing the NIR light energy or area, the cell detached area on the conductive polymer surface could be increased. These CP surfaces could be used for repetitive culture and detachment of MSCs in the same region. Cells *in situ*, within organ and tissue which comprised of several different cell types, continuously interact with neighboring cells *via*

paracrine signal processes to regulate their intrinsic functions. Therefore, to develop the cell matrix, in which the interactions with neighboring cells could be temporally and spatially controlled *in vitro*, is very important to better understand and approach strategies to control dynamic biological processes. However, most previous studies regarding light induced cell detachment utilized irreversible photoreaction, which made it impossible to perform the following characterization after first light exposure. As shown in this study, the cell harvesting using reusable CP-coated surface make it possible for dynamic multiple cell culture system, which allows to perform the following studies such as the effects of second cell type on remained cells after the detachment of first cell type. In particular, this reusable PEDOT substrate would be useful to study on stem cell differentiation where complicated but precisely controlled niche effectors were typically involve in for the long-term culture periods. Importantly, the detached MSCs by NIR triggering preserved their intrinsic characteristics and multilineage differentiation capacities. Therefore, we believe that our method for spatial and temporal control of cell detachment provides a novel possibility for the study of intercellular interactions, cell analysis, and design of engineered tissue. Furthermore, this is applicable to the delicate cell cloning, selective enrichment or depletion of specific cell(s), and can provide a simple and inexpensive way to remove contaminating agents (proteins, bacteria and cells) from the surface of various medical and dental devices.

EXPERIMENTAL SECTION

Materials and Instruments. 3,4-Ethylenedioxythiophene, iron-(III) *p*-toluenesulfonate, anhydrous isopropyl alcohol, and lithium perchlorate were purchased from Aldrich Chemicals and used without further purification. DMEM, fetal bovine serum (FBS), antibiotics (penicillin/streptomycin), phosphate-buffered saline (PBS, pH 7.4), trypsin/EDTA (0.05%), and Tryphan blue (0.4%) were purchased from Gibco (Invitrogen, Carsbad, CA). Ficoll-Hypaque and 7-AAD was purchased from GE Healthcare (Uppsala, Sweden) and Beckman Coulter (U.S.A.), respectively. The Live/Dead viability/cytotoxicity kit was purchased from Invitrogen (U.S.A.). Other chemicals and solvents were purchased from Aldrich (St. Louis, MO). The thickness of the polymer film was determined *via* an Alpha step profilometer (Tencor Instruments, Alpha-step IQ) with an accuracy of 1 nm. To confirm the PEDOT film morphology, PEDOT films were observed with a field emission-scanning electron microscope (HITACHI S-800, Tokyo, Japan) and the image was taken by a scanning microscope image analysis system (ESCAN-4000, Bummi Universe, Tokyo, Japan). A cyclic voltammogram was performed using a CHI624B potentiostat (CH Instruments, Inc.). UV/vis/NIR spectra of PEDOT films were obtained using a UV/vis/NIR spectrometer (Lambda 750, PerkinElmer). The optical MSCs images were obtained using an Olympus inverted research microscope (Model IX71). NIR coherent diode laser (808 nm, 230 mW, beam diameter size of 3 mm, B&W Tek, Inc.) was utilized to investigate the temperature increase of PEDOT based on photothermal effect and detach target cells on PEDOT surfaces. High power NIR diode laser (808 nm, 1670 mW, beam diameter size of 10 mm, UM30K, Jenoptik) was used for the study on lateral thermal diffusion and spatial resolution of cell detachment (Figures S3 and S10). We have first investigated the temperature increase on PEDOT thin film by changing a range of laser power to find a minimum laser power for temperature change just enough for cell detachment without invoking cell viability. Temperature increase based on photothermal effect was relatively weak at low irradiation power (<230 mW). Therefore, the minimum power was 230 mW in our study, for efficient photothermal effect and cell detachment.

Preparation of PEDOT Thin Film Substrates through Solution Casting Polymerization (SCP). Fe(III) tosylate (1.07 g) was dissolved in isopropyl alcohol (1.85 g) and stirred for 1 h at room temperature. To this solution, 0.06 g of pyridine was added and stirred for 1 h and filtered by a hydrophilic syringe filter. Monomer (0.1 g) was added to this oxidant solution, stirred for 5 min, and then spin-coated on substrates at 2500 rpm for 40 s. The solution-coated substrate was heated to 60−80 °C to induce oxidative polymerization and produce a colored polymer film on the substrate. After cooling to room temperature, the conductive polymer-coated substrate was washed several times with isopropyl alcohol to remove residual oxidant, low molecular weight oligomers, and impurities. The film was dried under nitrogen flow and used as a cell culture substrate. The thickness of the resulting PEDOT film was 160−180 nm. PEDOT films containing different film thicknesses were prepared by changing the spin coating speed. To prepare partially doped and dedoped PEDOT surfaces, +0.4, −0.2, and −1 V was applied to PEDOT-coated ITO glass in a three-electrode electrochemical cell consisting of a stainless steel as a counter electrode and Ag/AgCl as a reference electrode in the presence of 0.1 M $LiClO_4$ in acetonitrile (ACN) as a electrolyte solution. These PEDOT films (SCP-PEDOT, partially doped PEDOTs, and neutral PEDOT) were used for spectroscopic measurements, photothermal effects, biocompatibility, proliferation, and NIR-induced detachment of MSCs.

Measurement of the Photothermal Effect of Conductive Polymer (PEDOT) Thin Films. PEDOT films were prepared as previously mentioned. The PEDOT films were placed in a six well plate that was filled with cell media (DMEM, 3 mL). A thermocouple was directly placed in contact with the PEDOT films and separated by 3 mm from the NIR coherent diode laser (808 nm, 230 mW, beam diameter size of 3 mm, B&W Tek, Inc.) to prevent the effect of direct NIR irradiation on the temperature increase. The temperature of the sample was directly measured by a thermocouple (TES2732, Multimeter) with a minimum detection time of 1 s and a minimum threshold of 0.1 °C. It is important to note that temperature increase by NIR exposure at the area within the beam diameter (3 mm) is greater than that outside of the diameter.

Spatial Resolution Test of Photothermal Cell Detachment. As shown in Figure S10, the optical setup was equipped with iris diaphragm (OptoSigma, 089−1140) for the spatial resolution test of photothermal cell detachment on conductive polymer. The size of NIR laser beam (808 nm, 1670 mW, UM30K, Jenoptik) was controlled by iris diaphragm hole size from 0.5 to 7 mm. Cell detachment was carried out at 100% cell detachment efficiency condition (SP thickness = 165 nm; laser wavelength = 808 nm; NIR laser power = 1670 mW, 501 J).

Cell Culture. Bone marrow aspirates were collected from healthy donors with approval from the Research Ethics Committee of Severance Hospital in Yonsei University, Seoul, Korea (Approval No. 4−2008−0643) and informed consent. Bone marrow-derived MSCs were isolated and cultured as previously described.[26,27] In brief, mononuclear cell fraction was isolated by Ficoll-Hypaque density gradient centrifugation and plated in DMEM supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg mL^{-1} streptomycin) at a density of 10^5 cells cm^{-2} in a 75 cm^2 tissue culture flask (Nunc, Denmark) at 37 °C in 5% humidified CO_2. Nonadherent cells were removed after 24 h by exchanging culture medium upon repeated washing with PBS. The medium was changed every 3 or 4 days. On reaching 90% confluency, MSCs were recovered using 0.05% Trypsin/EDTA

and replated at a density of approximately 10 000 cells cm^{-2} in 15 mL of media as passage 1. Subsequently, cells were kept in culture for further expansion.

Proliferation Assay of MSCs on PEDOT Film Substrates. PEDOT films were prepared as previously explained and a PDMS mold (3×3.5 cm^2) was attached to the PEDOT films to preserve the same area. The prepared PEDOT film substrates were washed with media and placed in a 100 mm culture dish (Corning Inc. Corning, NY). Cells were harvested by incubation with 0.05% Trypsin/EDTA for 3−5 min at 37 °C, and seeded to the PEDOT film substrates at 6000 cells cm^{-2}. Cells were also cultured on a polystyrene tissue culture plate with a PDMS mold (3×3.5 cm^2) as a reference experiment. DMEM (4 mL) was added to each well and exchanged the next day. Cells were maintained for 3 days and then harvested for cell counting. The cell number was examined at three different times. The growth rates for MSCs on the sample after 3 days of culture were determined by counting the number of cells with a hemacytometer after Tryphan blue staining in a counting chamber.

7-AAD (7-Aminoactinomycin D) Staining. To determine apoptosis rates, 7-AAD (Beckman coulter) reagent was added to 1×10^5 cells in PBS. The cells were stained for 20 min at 4 °C in the dark, followed by washing with PBS. Samples were immediately analyzed using flow cytometry (Beckman Coulter).

Propidium Iodide (PI) Staining. To examine cell viability, with PBS. A 50 μg mL^{-1} propidium iodide reagent (Sigma Aldrich) was added to the cells and immediately imaged with a fluorescence microscope (Olympus IX-71).

Live-Dead Staining of Detached MSCs by NIR Exposure. The viability of MSCs detached by NIR exposure was analyzed by using the Live/Dead viability/cytotoxicity kit (Invitrogen). Briefly, harvested MSCs were incubated with phosphate-buffered saline (PBS) containing 2 μM of calcein AM solution and 4 μM of ethidium homodimer-1 solution at room temperature for 30 min. After rinsing with PBS, images were acquired using an Olympus inverted research microscope (Model IX71).

Western Blotting. Total protein was extracted using PRO-PREP (iNtRON, Seongnam, Korea). The proteins were separated by 10% gradient-precast sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto PVDF membranes (Invitrogen). The membrane was incubated with the primary antibodies; actin (Santa Cruz, CA, 1:1000), caspase-3 (Santa Cruz, CA, 1:400), heat shock protein-70 (Santa Cruz, CA, 1:300), and then with HRP-conjugated antibodies against goat IgG (Santa Cruz, CA, 1:1000). The secondary antibodies were detected by using LAS4000 mini (GE Healthcare).

Differentiation Assay. To induce osteogenic, adipogenic, and chondrogenic differentiation, cells detached by NIR exposure were seeded in differentiation media (Cambrex, Lonza, U.S.A.) for three weeks, with a media change every 3 or 4 days. When the media was changed for chondrogenesis, 10 ng mL^{-1} TGF-β3 was added to the media. After three weeks, cells were analyzed for osteogenesis, adipogenesis, and chondrogenesis using von Kossa, Oil red-O, and Safranin-O staining, respectively. The stained cells were imaged with a phase microscope (Olympus IX-71).

Immunophenotyping. Cells for analysis were trypsinized and placed into FACS tubes at 3×105 cells per tube. After washing with PBS, the cells were incubated at 4 °C for 20 min with antibodies (CD34-FITC, CD29-FITC, CD90-FITC) in the dark. IgG1-FITC was also used as a negative control. The cells were then washed with PBS and fixed with 1% (w/v) paraformaldehyde in PBS for FACS analysis. Then, cells were investigated with a Cytomics Flow Cytometer (Beckman Coulter).

Statistical Analysis. Mean and standard deviation were calculated and results were expressed as mean ± SD of multiple determinations. Comparisons between groups were evaluated by two-side Student's *t* test. The minimum level of statistical significance was set at $p < 0.05$.

Conflict of Interest: The authors declare no competing financial interest.

Supporting Information Available: Thermal image of PEDOT film, lateral thermal diffusion, optical microscopic images of MSCs on TCPS and PEDOT surfaces, FE-SEM, 7-ADD analysis, PI staining, FT-IR, CV, spatial resolution of cell detachment, immunophenotyping. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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