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| Original Article |

Fast and Efficient Isolation of Mouse Bone Marrow-Derived Mesenchymal Stem Cells by Using a Biocompatible Polymer

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Abstract : Mesenchymal stem cells (MSCs) differentiate into bone, fat, cartilage, tendon, and other organ progenitor cells. The rarity of MSCs in bone marrow necessitates fast and efficient isolation and/or *in vitro* expansion prior to clinical and biomedical applications. Previously, we reported that UV-exposed diphenylamino-s-triazine bridged *p*-phenylene vinylene (DTOPV-UV) with a hydrophilic and negative surface-containing carboxyl group is highly biocompatible and provides a substrate for efficient human bone marrow-derived MSC attachment. In this study, we applied this polymeric film to early adhesion and enrichment of MSCs from mouse bone marrow. With its high protein-binding capacity, DTOPV-UV film was more efficient in early capture of adherent bone marrow cells than conventional tissue culture polystyrene (TCPS). Cell binding to DTOPV-UV reached full capacity within 1 hr, whereas cell attachment to TCPS gradually increased over time. The isolated and culture-expanded MSCs from mouse bone marrow displayed typical morphology, phenotype, and differentiation into osteoblasts, adipocytes, and chondrocytes. Here, we demonstrate a novel method for isolating MSCs from mouse bone marrow using a biocompatible polymer. This method will aid the development of rapid and efficient isolation and *in vitro* expansion protocols for rare adherent cells.

Key words: *adhesion, mesenchymal stem cell, bone marrow, carboxyl group*

1. Introduction

Bone marrow contains a rich supply of at least three different stem cells: hematopoietic stem cells, mesenchymal stem cells (MSCs), and endothelial progenitor cells. Of these, MSCs are a rare population of multipotent stem cells (0.01% to 0.001% of bone marrow mononuclear cells) with the ability to self-renew and differentiate into several distinct mesodermal,[1] ectodermal,[2-3] and endodermal[4] lineage cells. Because of their versatility, MSCs have received considerable attention for use in clinical applications in regenerative medicine.

A key feature of bone marrow-derived MSCs is their ability to adhere to tissue culture plastic. This feature, which was described four decades ago, is the gold standard procedure for MSC isolation.[5-6] Although mouse MSCs are a good model for preclinical investigations, their isolation and expansion from mouse bone marrow cells based on plastic adherence has proven to be less than successful due to the high content of hematopoietic cells in bone marrow and the unwanted growth of non-MSCs in primary culture of the harvested bone marrow.[7] Therefore, alternative isolation methods for isolating mouse bone marrow MSCs are the subject of intensive investigation. Other than adhesion, MSCs have been isolated by positive selection[8] or negative depletion[9] with microbead-conjugated antibodies, by density, by media composition, and by other capture molecules including antibodies and extracellular matrix proteins.[9-10] Despite these efforts, new methods for fast and efficient isolation of MSCs are still required to overcome the limitations by enhancing the attachment of MSCs to the desired substrate, and promoting their expansion. In addition to the difficulties encountered during isolation, MSCs age quickly and lose their potency during *in vitro* culture. Since the interaction between stem cells and the microenvironment is important for the maintenance of stem cell characteristics,

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development of a supporting substrate that provides a good microenvironment for isolation and expansion of MSCs with long-term maintenance of stemness is critical.

Recently, the interaction between stem cells and biocompatible polymers has become an important issue in the biomedical arena and is of interest in the fields of stem cell biology, tissue engineering, diagnostics, microfluidics, and gene and drug studies.[11] Ideally, polymers with different chemical compositions,[12-13] surface charges,[14] wettabilities,[15] and morphologies[16] enable us to select specific subsets of cells and fine tune their behaviors, including proliferation, differentiation and apoptosis, thereby exploring the possibilities of modern cell engineering. Thus, modifying the surface properties is of great importance for controlling protein and cell attachment. Although the mechanism of cell attachment to polymer surfaces has been extensively studied,[17] this complex process is not fully understood. In addition, little is known about the application of polymer substrates to stem cell isolation and expansion.

In a previous study, we found that UV-exposed diphenylamino-s-triazine bridged *p*-phenylene vinylene (DTOPV-UV) with a hydrophilic and negative surface-containing carboxyl (-COOH) group is highly biocompatible and provides a good substrate for efficient human bone marrow-derived MSC attachment.[18] This finding prompted us to investigate the role of this substrate in the rapid isolation and culture of MSCs from mouse bone marrow. We hypothesized that a cell adhesion-supportive polymer provides a good microenvironment for fast and efficient MSC attachment, which are crucial for their survival in the harsh competing microenvironment between rare MSCs and other hematopoietic bone marrow cells.

2. Materials and Methods

2.1 Reagents and Chemicals

DMEM with low glucose, fetal bovine serum (FBS), penicillin/streptomycin, L-glutamine, PBS, trypsin/EDTA (0.05 %), 1x insulin-transferrin-selenium (ITS), and trypan blue (0.4 %) were purchased from Invitrogen (Carlsbad, CA, USA). Human serum albumin (5%) was purchased from Green Cross Corporation (Korea), and 7-amino-actinomycine D (7-AAD) was obtained from Beckman Coulter (USA). TGF-β was purchased from Peprotech (USA). DAPI (4’-6-diamidino-2-phenylindole), formalin, paraformaldehyde, glutaraldehyde, crystal violet, silver nitrate, Oil Red O, Safranin-O, and fibronectin from human plasma were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 Fabrication and Evaluation of Surface Properties of DTOPV-UV

DTOPV was synthesized via Wittig polycondensation as reported previously.[18] Briefly, DTOPV film with an average thickness of 140 nm was prepared by spin coating with a chloroform solution of DTOPV (1 wt %) at 13000 rpm for 15 sec and then dried for solvent removal. DTOPV film was illuminated with a high-intensity UV lamp (13.05 mW/cm^2) for 30 min and designated as DTOPV-UV. Wettability of the polymer surface was investigated by determination of DI water drop contact angle measurements at ambient conditions (20°C, 30~40% humidity). Atomic force microscopy (AFM, Dimension 3100 SPM equipped with Nanoscope Iva; Digital Instruments, USA) was employed to observe the surface structure of the substrates as described previously.[18] To visualize cellular attachment to substrate, cells on DTOPV-UV were fixed with 2.5% glutaraldehyde, subsequently dehydrated with increasing concentrations of ethanol (50, 70, 80, 90, 95, and 100%), and critical-point dried. The samples were then coated with a thin layer of platinum-palladium and observed with a field emission-scanning electron microscope (SEM, HITACHI S-800, Japan).

To determine the direct cytotoxicity of the polymer-coated plates, mouse MSCs cultured on TCPS or DTOPV-UV for 48 hr were detached by trypsin/EDTA, washed with PBS, and stained with the vital dye 7-AAD for 10 min. An accumulation of 7-AAD fluorescent dye was quantified by flow cytometry.

2.3 Protein Adsorption of Human Serum Albumin and Fibronectin to DTOPV-UV and TCPS

The protein-binding capacity of the polymer substrates was determined by ELISA. Briefly, 10 μg/ml of human serum albumin or fibronectin was added to the TCPS- or DTOPV-UV-coated wells and incubated at 37°C for 1 hr. The non-adsorbed fraction was harvested and quantitated by human fibronectin ELISA kit (AssayPro, Winfield, MO, USA) and human albumin ELISA kit (Komabiotech Seoul, Korea) according to the manufacturer’s instructions. The surface density of albumin and fibronectin (expressed as ng/cm^2) was determined from the surface area of the wells after correcting for the non-adsorbed fraction.

2.4 Mouse Bone Marrow Cell Culture

All animal procedures were approved under the guidelines of the Health Sciences Animal Policy and Welfare Committee of the Yonsei University College of Medicine. Balb/c and C57BL/6 mice that were 6-8 weeks old (Orient Bio, Korea) were sacrificed by cervical dislocation, and their femurs and tibia

were carefully cleaned from adherent soft tissue. The tip of each bone was removed with a rongeur, and the marrow was harvested by inserting a 26-gauge 1-ml syringe needle (Korea Vaccine Co. Ltd, Korea) into one end of the bone and flushing with culture medium (DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, and 1% L-glutamine). After filtering through 40-μm nylon mesh filter (BD Falcon, USA), 1×10^7 cells were immediately plated into TCPS- or DTOPV-UV-coated wells (length×width×height = 2 cm×2 cm×1 cm) in the culture medium and cultured under standard cell culture conditions (37°C in a humidified 5% CO2/95% air atmosphere). At the end of the incubation period of 1, 3, or 6 hr, the supernatants and unbound cells were aspirated, and the wells were gently washed three times with 1 ml PBS to remove the non-adherent cells.

2.5 Quantification of Cell Attachment

To examine the cells attached to the substrate, the cells were washed with PBS with 0.1% BSA and fixed in 4% paraformaldehyde at room temperature. After 20 min, the cells were washed with wash buffer two times for 5 min each and then stained with DAPI (1 μg/ml in PBS) in the dark. Next, the cells were washed and photographed with a fluorescent microscope (Olympus-BX51). Cell adhesion was quantified by counting DAPI-positive nuclei in three microscopic fields obtained at 10x magnification from predetermined areas.

Alternatively cell adhesion was measured by crystal violet staining. After adhesion of bone marrow cells on TCPS and DTOPV-UV, medium was changed every 8 hr for 3 days, and the bound cells were cultured for an additional 5 days. Cells were fixed in 10% formalin for 30 min at room temperature and then stained with 0.05% crystal violet solution in distilled water. The stained cells were photographed with a microscope (Olympus-BX51). The amount of cell binding was quantified by measuring the absorbance of the crystal violet solution at 595 nm.

2.6 MSC Generation

For MSC generation, bone marrow cells were seeded onto TCPS or DTOPV-UV-coated wells at a density of 1×10^7 cells per well, and medium was changed every 8 hr for 3 days. When primary culture of MSCs became nearly confluent, the culture was treated with 0.5 ml of 0.25% trypsin/EDTA for 2-5 min. Harvested MSCs were seeded onto TCPS or DTOPV-UV-coated wells at a density of 4×10^4 cells per well. The medium was changed every 3 days. Typically, cell confluence was achieved after 7 days. Once the culture reached 70-80% confluency, the cells were harvested and expanded for further experiments.

2.7 Immunophenotyping

The detached MSCs were stained on ice for 30 min with the following anti-mouse monoclonal antibodies: anti-CD44, anti-CD45, anti-CD29, anti-CD71, anti-CD90, anti-CD106, and anti-CD117 (all from BD-Pharmingen, USA). After staining with primary antibody, secondary antibodies conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE; BD-Pharmingen) were used for the analysis. Cells were examined with a Cytomics™ Flow Cytometer (Beckman Coulter) and analyzed using WinMDI 2.8 software (http://facs.scripps.edu/help/html/read1ptl.htm). Cell viability was determined with 7-AAD staining followed by flow cytometric analysis.

2.8 Multipotency of MSCs Generated by DTOPV-UV-Coated Plates

Twelve-well cell culture plates (Falcon) were used for these assays. For multilineage differentiation, media purchased from Cambrex (Lonza, USA) was used. The cells for osteogenic and chondrogenic differentiation were plated at 5×10^4 cells per well. We utilized monolayer culture for chondrogenesis. The cells for adipogenic differentiation were seeded at 1×10^5 cells per well. Culture medium was used as an internal control. For chondrogenesis, monolayer of MSCs was cultured with medium containing 10 ng/mL TGF-β3. Cultures were incubated for 2 weeks in 95% air/5% CO_2 at 37°C, with fresh medium change every 3-4 days. Chondrogenesis was perfomed by staining with Safranin-O. Osteogenesis was demonstrated by accumulation of mineralized calcium phosphate assessed by von Kossa staining. Adipogenesis was confirmed by intracellular accumulation of lipid-rich vacuoles stained with Oil Red O.

2.9 Statistical Analysis

Values were averaged and expressed as means±standard deviation (SD). Statistical differences were determined by a Student *t*-test. The differences were considered statistically significant at $p < 0.05$.

3. Results

3.1 Surface Chemistry of DTOPV-UV

The biocompatible polymer film used for this study was a DTOPV polymer synthesized using a previously reported method.[19-20] The vinylene group of the DTOPV was changed to a carboxyl group by photo-oxidation upon exposure of the film to a UV source for 30 min (Fig 1). The water contact angle of untreated DTOPV film was 98° and was considered

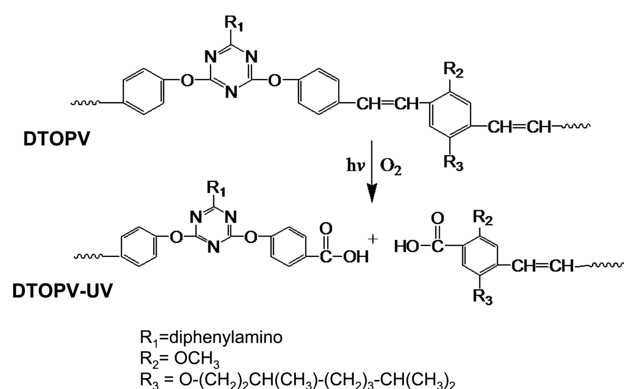

Figure 1. Structure and photo-oxidation reaction of diphenylamino-s-triazine bridged *p-phenylene vinylene* polymer (DTOPV).

Table 1. Characteristics of polymer surfaces

Substrate/contact angle image	contact angle -water
TCPS	60.5±1.4
Polystyrene	83.7±1.7
DTOPV	98.0±0.8
DTOPV-UV 30 min	92.1±0.1

hydrophobic, and UV illumination of DTOPV for 30 min changed the water contact angles to 92.1^{o} (Table 1). Although the hydrophilicity of the DTOPV surface was increased by UV illumination due to the formation of carboxyl groups,[18] the hydrophilic character of DTOPV-UV is not better than that of TCPS. Quantitative measurements of surface roughness were analyzed by AFM and SEM (Fig 2). The dry, DTOPV-UV-coated film was extremely smooth (peak-to-trough roughness of less than 1 nm, root mean square (rms) roughness = 0.440 nm) and had no evident surface features. The rms roughness of TCPS was 8.288 nm. The same trend was observed in the SEM images of MSC culture (Fig 2C). Thus, the smooth and featureless surface of DTOPV-UV is suitable for cell adhesion.

Next, we investigated the capacity of protein adsorption on the surface of DTOPV-UV and TCPS by ELISA, as adsorption is the first event during contact of cells to a polymer surface in a culture medium containing proteins (Fig 3A). No significant difference in the amounts of albumin that adsorbed to the DTOPV-UV film and TCPS (882±151 ng/cm^2 and 632±24 ng/cm^2, respectively) were observed. In contrast, DTOPV-UV adsorbed significantly more fibronectin than the TCPS surface (1,334±186 ng/cm^2 and 857±86 ng/cm^2, respectively). These results suggest that hydrophobic and electrostatic interactions may be involved in the adsorption of extracellular matrix proteins to the DTOPV-UV film. This polymer coating exhibited little cytotoxicity to MSCs (3.7±1.1% 7-AAD$^+$ cells) in comparison to TCPS (3.1±0.9% 7-AAD$^+$ cells), suggesting that DTOPV-UV is

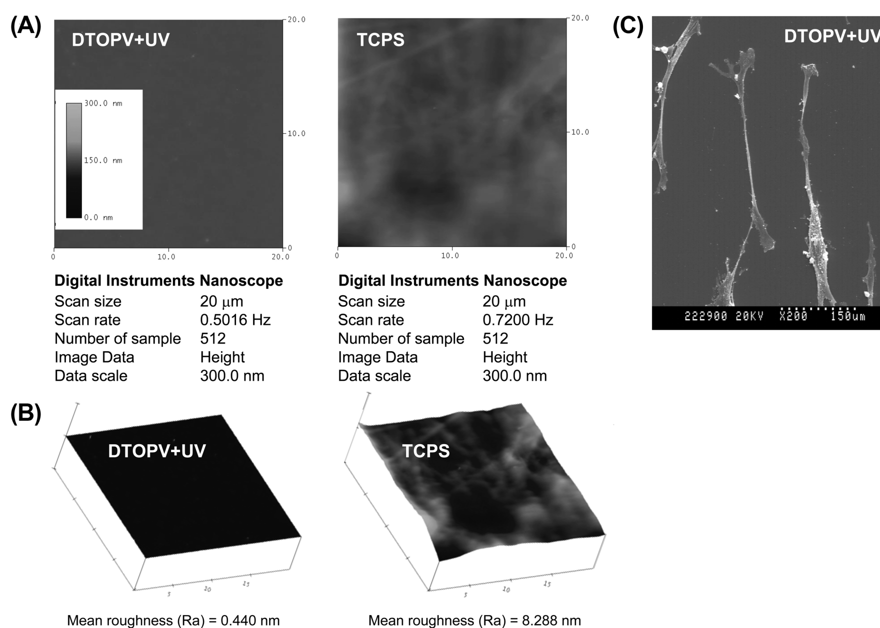

Figure 2. Representative AFM topography of DTOPV-UV and TCPS. (A) Two-dimensional and (B) three-dimensional AFM images of DTOPV-UV and TCPS surfaces are shown. (C) SEM image of MSCs on DTOPV-UV film also confirms the smooth and featureless surface of DTOPV-UV (150 μm line width).

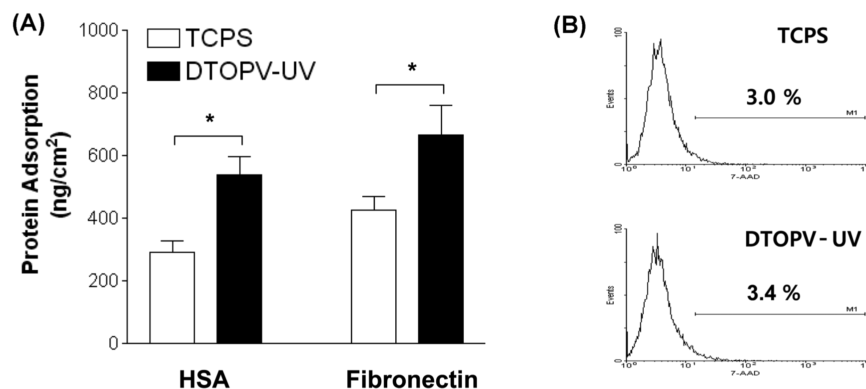

Figure 3. Protein adsorption of human serum albumin and fibronectin to DTOPV-UV and TCPS. (A) Human serum albumin and fibronectin were examined. DTOPV-UV film- and TCPS-coated wells were incubated with 10 μg/ml of albumin and fibronectin for 1 hr at 37°C. Surface concentrations of adsorbed protein (ng/cm^2) were calculated from the values of the unadsorbed fraction. *$p < 0.05$, n=3. (B) Cytotoxicity of DTOPV-UV on MSCs. Equal numbers of mouse MSCs were cultured on either TCPS- or DTOPV-UV-coated plates for 48 hr. Cells were then harvested and stained with 7-AAD. The number of cells accumulating 7-AAD was determined by flow cytometry.

suitable for use in cell culture (Fig 3B).

3.2 Fast Adherence of Bone Marrow Cells to DTOPV-UV

We examined whether the DTOPV-UV polymer could be applied to the isolation of mouse MSCs from bone marrow under the assumption that the DTOPV-UV film provides a good microenvironment for efficient attachment of mouse MSCs. We found that the attachment of bone marrow cells to DTOPV-UV for 3 hr was 2.1-fold greater than the attachment of these cells to TCPS (Fig 4). We also examined the optimal duration of adherence for the isolation of MSCs on DTOPV-UV. Unbound cells were removed by aspiration after 1, 3, or 6 hr, and bound cells were cultured in fresh medium. The number of cells bound to DTOPV-UV increased with incubation time (Fig 5). The attachment rate of the cells cultured on DTOPV-UV was much higher than that of cells cultured on TCPS at all time points tested. These results indicate that DTOPV-UV is a highly efficient substrate for the isolation of MSCs from mouse bone marrow.

3.3 Adherent Bone Marrow Cells to DTOPV-UV Exhibit MSC Characteristics

On TCPS, adherent spindle-shaped cells appeared as individual cells on days 3-4, and the cultures became 70% confluent within 2 weeks. While the first appearance of MSC-like cells on DTOPV-UV was not significantly different from that on TCPS, the culture reached 70% confluence within 10 days due to the higher initial progenitor contents. The resulting cells exhibited typical MSC morphology by crystal violet staining (Fig 6A). To examine the quantity of attached MSC-like cells, we performed ELISA and verified total cell number indirectly by absorbance of stained cells. In the result, the greatest number of MSC-like cells was generated when the medium was changed after 6 hr (Fig 6B). Thus, the initial interaction between polymer and MSCs during the first 6 hr of culture greatly increased the chance of binding and expansion of MSCs. Unlike DTOPV-UV, recovery of MSC-like cells on TCPS was not significantly improved after 6 hr of incubation compared to 1 and 3 hr of incubation.

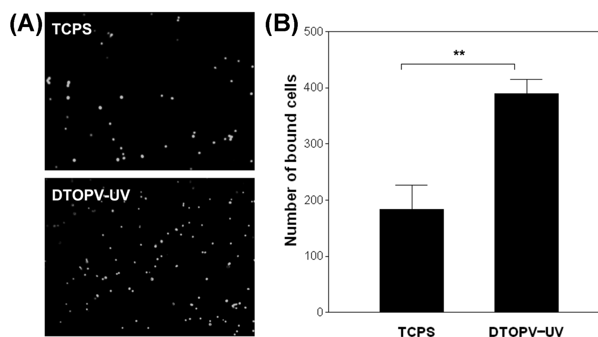

Figure 4. Adhesion of bone marrow cells to TCPS and DTOPV-UV. (A) Attached cells were visualized by DAPI stain. After 3 hr of incubation with 1×10^7 bone marrow cells per well (length×width×height, 2×2×1 cm), unbound cells were removed by aspiration, and bound cells were stained with DAPI. The stained nuclei (white dots) were photographed at magnification of ×100. One representative field of three independent experiments is shown. (B) The numbers of bound cells were counted in three fields. The error bars representing±SD. **$p < 0.01$, n=3.

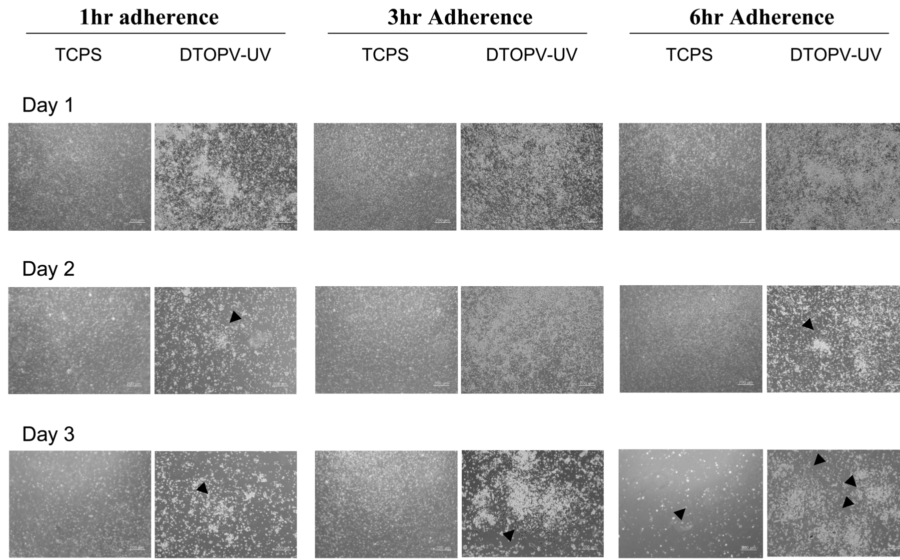

Figure 5. Efficiency of bone marrow cell binding to DTOPV-UV at different time points. Equal numbers of bone marrow cells (1×10^7 per well) were seeded onto TCPS and DTOPV-UV. After 1, 3, and 6 hr, media was changed, and adherent cells were cultured for 3 days. Clusters of bone marrow cells with MSC-like morphology are indicated by arrowheads (magnification, ×100).

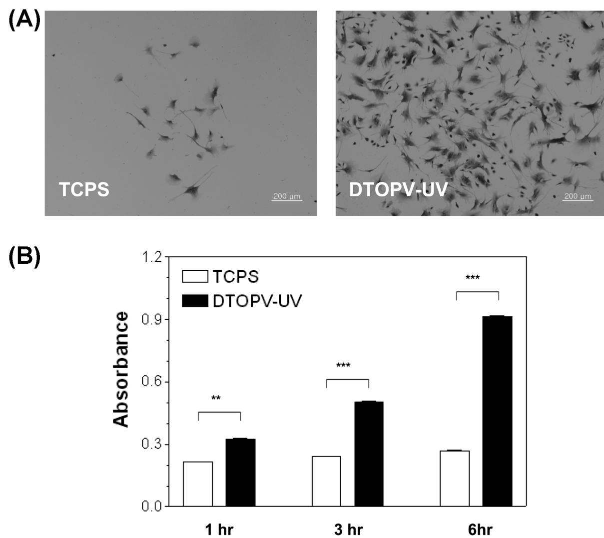

Figure 6. Generation of MSC-like cells from attached cells. Cells were cultured as described in Materials and Methods. (A) On day 8, adherent cells were stained with crystal violet. The cells displayed typical spindle-shaped MSC morphology. (B) Bound cells (crystal violet-stained cells) were quantified by absorbance at 595 nm. *$p < 0.05$, **$p < 0.01$ and ***$p<0.005$, n=3.

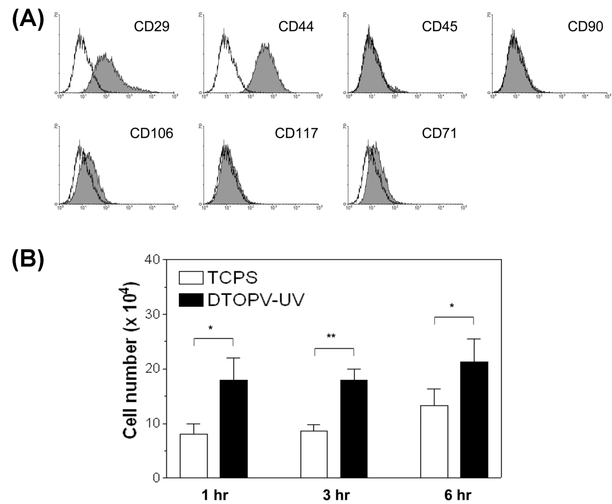

Figure 7. Characterization of cells generated from cells that adhered to the DTOPV-UV surface. Immunophenotyping of cells generated from the adherent bone marrow cells on the DTOPV-UV polymer surface exhibited the typical pattern of MSCs. Histogram of MSCs stained with anti-CD29, CD44, CD45, CD71, CD90 (Thy-1), CD106 (VCAM-1), and CD117 (c-kit) are shown with an overlaid isotype control. (B) Kinetics of MSC adherence to DTOPV-UV and TCPS. Equal numbers of bone marrow-derived MSCs were cultured for the indicated times, and the numbers of bound cells were enumerated. *$p < 0.05$ and **$p < 0.01$, n=3.

The morphological homogeneity of the generated MSCs (at passage 2) was apparent by flow cytometric analyses. MSCs exhibited a typical forward scatter and side scatter plot. MSCs from BALB/c mice were positive for CD29 and CD44 and negative for CD45, CD71, CD90, CD105, and CD117 antigens (Fig 7A). The morphological properties, including the shape,

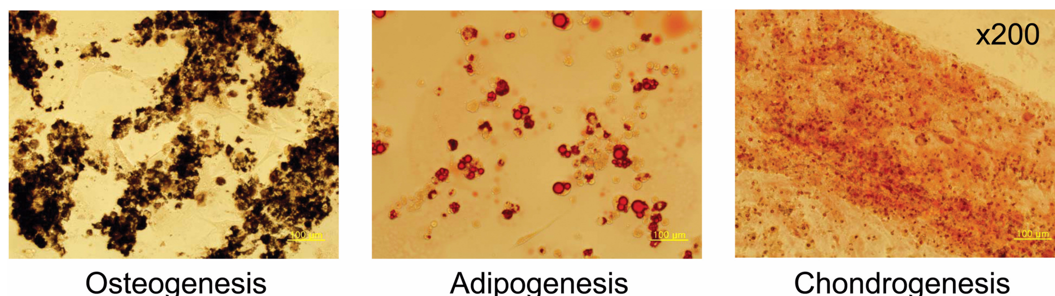

Figure 8. Differentiation potential of MSCs generated from adherent bone marrow cells on DTOPV-UV-coated plates. Osteogenic, chondrogenic, and adipogenic differentiation of MSCs generated from adherent bone marrow cells on DTOPV-UV was examined after three passages as described in the Materials and Methods. (Magnification, ×200)

spreading, and viability, of MSCs on DTOPV-UV were comparable to that of MSCs on TCPS, indicating that DTOPV-UV is a biologically inert polymer. Next, we examined the ability of DTOPV-UV and TCPS to support MSC adhesion. The direct counting of adherent cells revealed that DTOPV-UV supported fast adherence of MSCs (Fig 7B). On average, the number of attached cells to DTOPV-UV was more than twice that of TCPS at 1 hr. In contrast to MSC adherence to TCPS, which showed a gradual increase in the number of bound cells with longer incubation, DTOPV-UV reached saturation of adherence after 1 hr of incubation, implying that this substrate provides a better microenvironment for fast MSC attachment.

3.4 DTOPV-UV Efficiently Supports Self-Renewal and Multipotency of MSCs

In order to confirm the multipotential properties of mouse MSCs generated by DTOPV-UV, we assayed for adipogenesis, osteogenesis, and chondrogenesis in vitro (Fig 8). Cells expanded on the surface of DTOPV-UV displayed unaltered multipotency characteristic of MSCs as compared to cells on TCPS (data not shown). Adipogenic differentiation of MSCs after 2-3 weeks incubation formed scattered red vacuoles stained red with Oil Red O, whereas cells under osteogenic culture condition exhibited a homogeneous and relatively abundant number of von Kossa-positive cells within the same time limit. In addition, chondrogenic differentiation was evidenced by safranin-O staining. These results demonstrate that the matrix provided by DTOPV-UV biopolymer is fully supportive of the multipotency of MSC as well as of self-renewal.

4. Discussion

MSCs, multipotent progenitor cells of adult tissues, have the capacity to differentiate into various lineages *in vitro* as well as *in vivo*.[1,21] In addition, recent studies have shown that these cells possess an immunoregulatory property.[22-23] Due to the versatility of MSCs, increasing attention has been paid to this cell type. While plastic adherence is the primary choice for the isolation and enrichment of MSCs from human and experimental animals, this substance has proven unsuccessful for mouse MSCs because of the low MSC frequency and contamination of hematopoietic cells in the culture.[24] To overcome this limitation, we developed a new technique based on the biocompatible polymer DTOPV-UV.

Development of polymer coatings has emerged as one of the most active areas in biomedical technology in recent years. Studies have demonstrated that functional groups on the surface of polymer are of great importance for cell adherence and protein adsorption.[25] Although the complex process of cell binding to the surface of polymer is not fully understood, it is evident that manipulation of the surface chemistry of polymers can actively influence protein-to-surface interactions as well as cell-to-surface interactions. This manipulation, can, thus lead to changes in cellular behavior such as attachment, proliferation, apoptosis, and differentiation. We previously showed that DTOPV-UV has a hydrophilic and negative charge and supports adherence of MSCs efficiently.[18] In the present study, we focused on the short-term adhesion (from 1~6 hr) of mouse bone marrow cells to polymer-coated substrate in order to enrich bone-marrow-derived MSCs. We showed remarkably high cell attachment during this limited time window compared to attachment to conventional TCPS. While the surface chemistry of the substrate is known to greatly affect human MSC differentiation,[26] knowledge is lacking about the selective isolation of MSCs via modification of surface chemistry of a given polymer.

The surface of DTOPV-UV, which retains more ECM proteins than TCPS, resulted in improvements in cell adhesion and proliferation. DTOPV-UV adsorbed 1.5-fold more

fibronectin than TCPS. The hydrophobic and electrostatic interactions may be responsible for the enhanced fibronectin adsorption to the hydrophobic surface of DTOPV-UV, since both DTOPV-UV and fibronectin are negatively charged at neutral pH. In line with others,[27-28] we previously showed that extracellular matrix proteins in the serum contribute to the attachment of cells to polymer-coated surfaces.[18] Unlike TCPS, adherence of cells to DTOPV-UV gradually increased during 6 hr of incubation, and this provides an increased chance of bone marrow cells binding to the substrate for survival and expansion. On the other hand, the binding kinetics of the generated MSCs reached saturation within 1 hr on DTOPV-UV. Binding of MSCs to TCPS, however, did not reach a plateau within 6 hr, implying that the surface of DTOPV-UV favors fast and efficient attachment of MSCs. This discrepancy in cell adhesion kinetics on DTOPV-UV may be due to differences in the cellular composition of bone marrow mononuclear cells versus homogenous MSCs.

On the DTOPV-UV plate, fibroblastic colonies were observed within 3~4 days after initial culture, and cell confluence was achieved after an additional 1~2 weeks of culture. Following isolation of MSCs, the rate of proliferation (doubling time) increased sharply on the polymer compared to the traditional TCPS. These results strongly suggest that the DTOPV-UV, which is a negatively-charged polymer surface containing a carboxyl group through a high capacity for ECM protein binding than TCPS provides a better microenvironment for increased efficient adhesion and proliferation of MSCs. As of DTOPV-UV, standard TCPS provides a homogenous negative charged surface composed of negatively charged carboxyl groups. Thus, the same polymer chemistry may have differential effects on the behavior of different proteins and/or cell types, and thus, a universal theory of protein adsorption and cell adhesion to polymer surfaces with different chemistries may not be attainable. For example, carboxyl groups exhibit a negative effect on cell adhesion and behavior of various cell types.[29-30] Indeed, this property may have contributed to the selective binding of MSCs to the DTOPV-UV in the presence of excessive numbers of competitors since the negatively-charged surface inhibits adhesion of the major adherent cells (such as monocytes and macrophages) from the bone marrow to DTOPV-UV.

In summary, we efficiently captured and cultured mouse bone marrow MSCs via their immediate adhesion to DTOPV-UV. Cultured MSCs expanded much more quickly on DTOPV-UV-coated surface than on conventional TCPS, and the resulting MSCs exhibited typical morphology and phenotypes and possessed equivalent multipotency. This study highlights the potential of using a biocompatible polymer to enrich a rare population of adherent cells for facilitating isolation, proliferation, and/or differentiation. This strategy for the relatively rapid isolation of MSCs is easily applicable to regenerative medicine with polymer-coated graft materials. Simple polymeric coating with different surface chemistries can be used to efficiently isolate stem or precursor cells from a heterogeneous mixture of cells in a relatively short amount of time and can be utilized as vehicles to deliver therapeutic cells to target tissues/organs. These biopolymers can also serve as a carrier or substrate for efficient cell attachment *in vivo* for tissue regeneration purposes and drug delivery or as a biosensing device. Finally, these biopolymers can also be used for *in vitro* diagnostic purposes, such as array chips for DNA/RNA, proteins, and cells/tissues.

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