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 endodermal4 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 selection8 or negative depletion9 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,
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. Ideally, polymers with different chemical compositions, surface charges, wettabilities, and morphologies 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, 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. 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 trypsin 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. 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/cm²) 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. 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/cm²) 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