Migration of Human Mesenchymal Stem Cells Using Corning® FluoroBlok™ Cell Culture Inserts

Application Note 484

CORNING

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Introduction

Mesenchymal stem cells (MSCs) represent a current and increasing area of research into improving the human condition. MSCs are multipotent cells that appear to have great therapeutic potential for repair of damaged tissues such as muscle and bone, and these cells have been differentiated into various mesenchymal tissues, including bone, cartilage, fat, muscle, and tendon.[1,2] MSCs have been demonstrated to respond to, and reside in, injured tissues;[1,5-7] however, the mechanisms controlling this process are not completely known.[1,2,6,7] As such, this is an area of current interest, specifically, the migratory response of MSCs toward various cytokines and growth factors.[1-10] One particular benefit of cell therapy using MSCs is the lack of ethical issues that are associated with the use of human embryonic stem cells, as MSCs may be easily obtained from adult bone marrow.[2-4,8,9]

In vitro migration assays can be used as model systems for measuring the directed movement of cells towards a chemoattractant stimulus, or to measure how a particular drug or antibody affects that movement. Through the use of Corning FluoroBlok cell culture inserts, easy quantitation of cell numbers post-migration is possible. This system consists of a light-tight polyethylene terephthalate (PET) membrane that efficiently blocks the transmission of light within the range of 400-690 nm, thus allowing fluorescence detection in a non-destructive manner. Once labeled cells migrate through the membrane, they can be detected using a bottom-reading fluorescence plate reader or microscope; cells present in the upper chamber of the insert are shielded from detection. The Corning FluoroBlok cell culture insert system enables both kinetic and endpoint migration assays, as well as multiplexing.

Materials and Methods

Cell Culture

Human mesenchymal stem cells (Lonza) were cultured in MSCGM™ (basal medium (MSCBM) supplemented with MSCGM SingleQuot™ Kit; Lonza). Every 3 to 5 days medium was refreshed or cells were passaged.

Migration Assay

Confluent P2-P4 cells were fed either fresh growth medium or TNF-α (1 ng/mL with 0.25% BSA) in basal medium overnight, then cells were trypsinized and counted. Migration was assayed in Corning FluoroBlok 24-multiwell insert plates (6.5 mm diameter) with 8.0 µm pores (Corning Cat. No. 351157). Prior to seeding MSCs in the insert, the apical side of the insert was coated with 0.1% (wt/vol) bovine gelatin for 1 hour at 37°C. Unstimulated or TNF-α-stimulated cells (40,000 cells in 200 µL migration medium) were seeded onto the apical surface of the insert (in triplicate).

Chemoattractants or controls were added to the basal chamber (600 µL in basal medium) as follows: 30% FBS (Life Technologies), positive control, 10 ng/mL recombinant human PDGF-AB, 150 ng/mL recombinant feline/human SDF-1β, 30 ng/mL recombinant human IGF-1, migration medium (basal medium with 0.25% BSA). All cytokines and growth factors were from R&D Systems.

Following overnight incubation at 37°C, 5% CO_2, cells were stained with 2.5 µM Corning calcein AM fluorescent dye (Corning Cat. No. 354217) for one hour at 37°C, 5% CO_2, then read on a bottom reading fluorescence plate reader (PerkinElmer EnVision™) at 485/535 nm (Ex/Em). Images were captured using an Olympus® IMT-2 fluorescence microscope with an Optronics® MagnaFire® digital microscope camera and Image-Pro Plus software.

Note: the data generated in this Application Note used the original (purple) FluoroBlok membrane. We have made improvements to this membrane since it was introduced. The new FluoroBlok insert uses a black membrane with improved spectral characteristics. General information in this Application Note applies to both versions of the product, but the specific wavelength ranges mentioned here apply only to the original (purple) version.

For details, see Technical Bulletin CLS-DL-CC-042: New PET Membrane for Corning FluoroBlok 3.0 µm and 8.0 µm Pore Size Cell Culture Inserts.

Results and Discussion

MSCs have previously been shown to migrate in response to specific growth factors or chemokine signals. It has also been demonstrated that TNF-α serves to increase MSC migration towards certain stimuli.[1,5-8,10]

As shown in Fig. 1, significant increases ($p < .05$, t-test) in chemotaxis response of MSCs were observed over basal conditions using PDGF-AB (TNF-α stimulated and unstimulated) and IGF-1 (TNF-α stimulated) but not SDF-1. Chemotaxis response seen withPDGF-AB was comparable to 30% FBS (positive control).

Unlike cumbersome alternatives that involve cell scraping or manual cell counting, Corning® FluoroBlok™ cell culture inserts offer an easy-to-use, quantitative detection method for studying cellular migration. Figure 2 shows the undersides of Corning FluoroBlok inserts with calcein AM stained mesenchymal stem cells following migration in response to various chemoattractants.

Acknowledgement

I thank Suparna Sanyal for her assistance in preparing this application note.

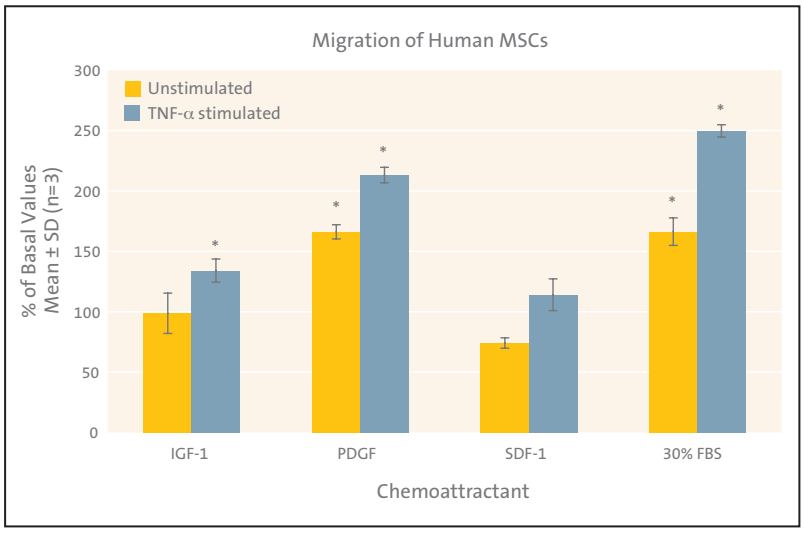

Figure 1. Migration of unstimulated and TNF-α-stimulated human MSCs towards chemoattractants. Overnight migration of MSCs through gelatin-coated Corning FluoroBlok inserts was allowed to take place in response to chemoattractants. Cells were stained with calcein AM and signals were read on a Perkin Elmer EnVision plate reader. Chemoattractants used were: 30 ng/mL IGF-1, 50 ng/mL PDGF-AB, 150 ng/mL SDF-1β, 30% FBS. RFU values of chemoattractants were compared to basal medium (MSCBM with 0.25% BSA) to generate percentages shown. Columns shown are representative data (n=3) from three experiments, mean ± SD,* $p<0.05$.

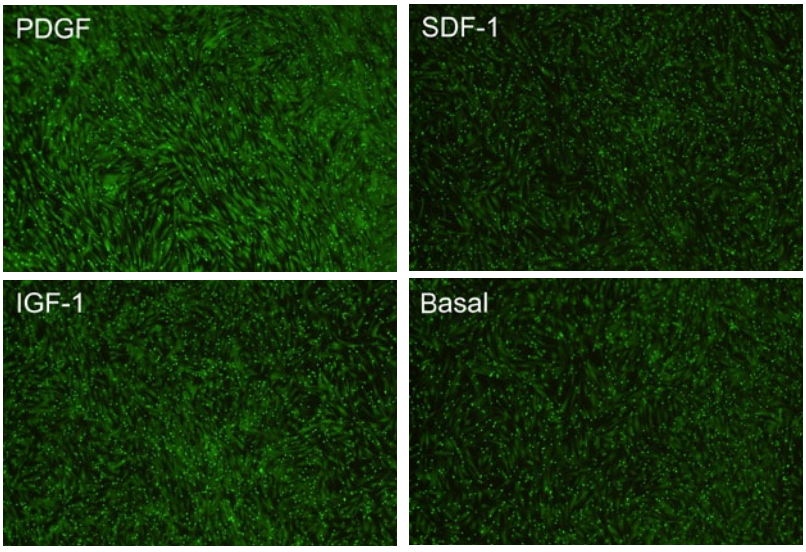

Figure 2. Calcein AM-labeled hMSCs. Undersides of representative Corning FluoroBlok inserts with TNF-α stimulated MSCs following migration toward chemoattractants or basal medium.

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