FLUXION

APPLICATION NOTE

Stem Cell Differentiation

Differentiation of mesenchymal stem cells under shear stress in the BioFlux system

Introduction

Stem cell research has the potential to produce novel treatments for previously incurable diseases and injuries. The application of controlled shear flow to undifferentiated embryonic stem cells promotes enhanced expansion of cell lines (Fok, E. and Zandstra, P., 2005). Shear stress is also used as a stimulus for differentiation especially for cell types that naturally respond to physiological shear, such as endothelial cells (Yamamoto et al 2003; Illi et al 2005; Wang et al 2005; Yamamoto et al 2005). Differentiation of cells into specific cell types and subsequent production of biomaterials is also facilitated by mechanical forces such as shear. This is the case with chondrocytes used to produce cartilage (Shuman et al 2006).

The BioFlux system (Figure 1) from Fluxion Biosciences delivers tightly controlled shear flow to biological samples. It utilizes BioFlux Plates which are microfluidic devices incorporated into SBS-standard well plate formats (Figure 2). The system is optimal for stem cell research, and enables the researcher to generate high content microscopy data while cells are growing under flow. The high density format permits the researcher to test up to 96 independent conditions simultaneously. Here, we demonstrate differentiation of mesenchymal stem cells under flow conditions into endothelial cells expressing vonWillebrand factor.

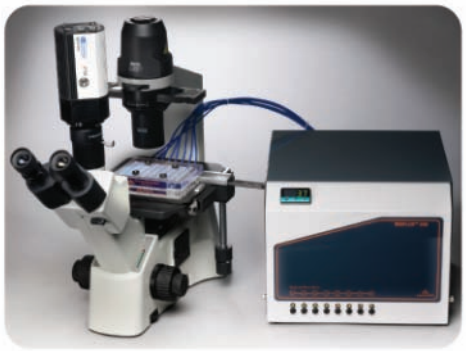

Figure 1: The BioFlux System for live cell assays under controlled shear flow.

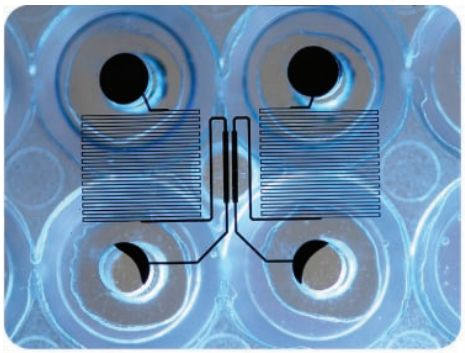

Figure 2: BioFlux Plate channels as viewed from beneath the well plate. Microfluidic flow cells are integrated into the bottom of an SBS-standard well plate. Each fluidic channel runs between pairs of wells and has a central viewing window for observation.

Methods

Cryopreserved (rat) mesenchymal stem cells (Millipore, Billerica, MA) were propagated as directed by the manufacturer. For BioFlux experiments, channels were coated with poly-l-lysine at room temperature for 1 hour and then washed with spent medium from the culture of the stem cells. Cells were seeded into the channels at 5 x 10^6 cells/ml and grown overnight by gravity flow in Mesenchymal stem cell expansion medium (Millipore). The next day, media was changed to CO_2 independent medium (Invitrogen, Carlsbad, CA) supplemented with either 10% FBS (SRM), 2%FBS (SFM) or 2%FBS with 50ng/ml recombinant rat vascular endothelial growth factor (VEGF) added (VEGF-M). An alternative to using CO_2 independent media would be to use the optional gas hookup to apply a 5% CO_2 gas mixture as the pressure source. The BioFlux Plate was placed on the heater plate; media were either perfused by gravity flow or at 1 dyn/cm^2 for 48-100 hours. Additional media was added as needed. Following perfusion, cells were fixed with 1% paraformaldehyde for 30 minutes, blocked and stained with a primary antibody (rabbit) against vonWillebrand factor (Abcam, Cambridge, MA) and Alexa 594 phalloidin (Invitrogen). Cells were washed with 0.5% BSA in PBS for 10 minutes at 1dyn/cm^2 and secondary antibody was added (anti-rabbit Alexa 488). Cells were washed in PBS for 10 minutes at 1dyn/cm^2 and Hoechst 33342 was added in PBS by gravity flow. Images were captured using a Nikon TS100 microscope and a CCD camera (QICAM)(Figure 3).

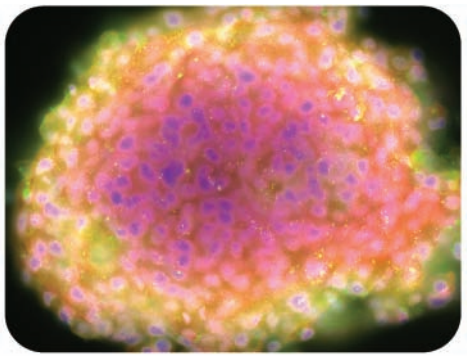

Figure 3: Mesenchymal stem cells differentiated under flow conditions for 48 hours in the presence of 10 % FBS (serum rich media).

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Results

Mesenchymal stem cells may be differentiated under different conditions into several types of specialized cells. Application of shear stress to mesenchymal stem cells or addition of VEGF may influence their differentiation to endothelial cells, which exclusively express and secrete vonWillebrand factor (vWF)(Oswald et al (2004) Stem Cells. 22:377; Zeng et al. (2006) JCB. 174:105). We used application of shear stress in the BioFlux system to differentiate mesenchymal stem cells into endothelial cells under flow conditions. We found that even after 48 hours of shear stress without addition of VEGF that cells began to express vWF in the cytosol. The most marked differentiation or expression of cytosolic vWF occurred without the addition of VEGF in rich media under shear (Figure 4, SRM-flow). However, differentiation also occurred in the VEGF/shear stress-treated cells as well (Figure 4, VEGF-flow).

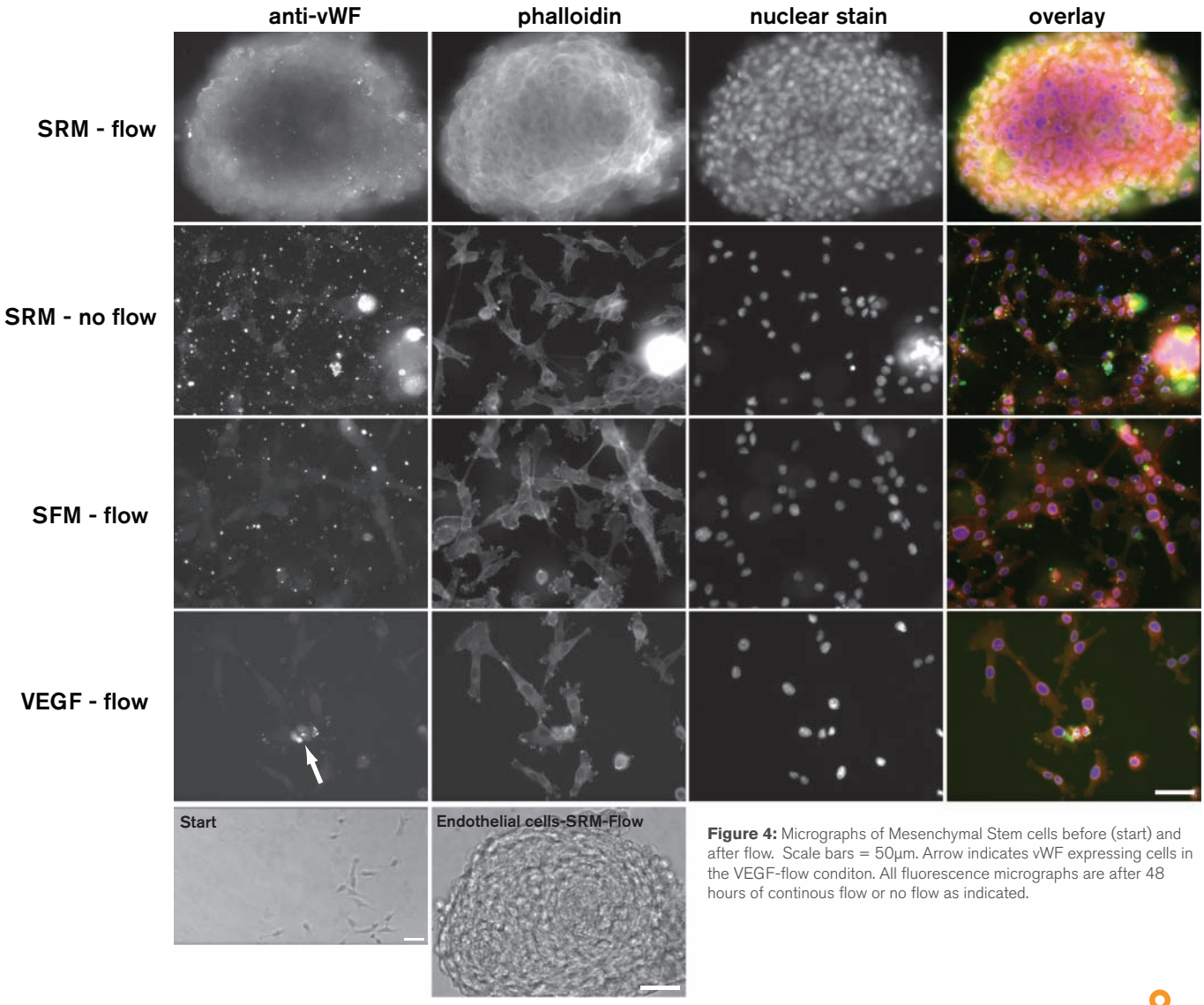

Figure 4: Micrographs of Mesenchymal Stem cells before (start) and after flow. Scale bars = 50µm. Arrow indicates vWF expressing cells in the VEGF-flow conditon. All fluorescence micrographs are after 48 hours of continous flow or no flow as indicated.

Summary

We have used the BioFlux system to differentiate stem cells under shear stress. The BioFlux system provides facile access to controlled cellular microenvironments including temperature, gas mixes and shear stress making it amenable to differentiation experiments and other specialized cell culture for stem cells and beyond.

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