CELL Microsystems®

APPNOTE

Stem Cell Differentiation

Differentiation of mesenchymal stem cells using shear stress

Introduction

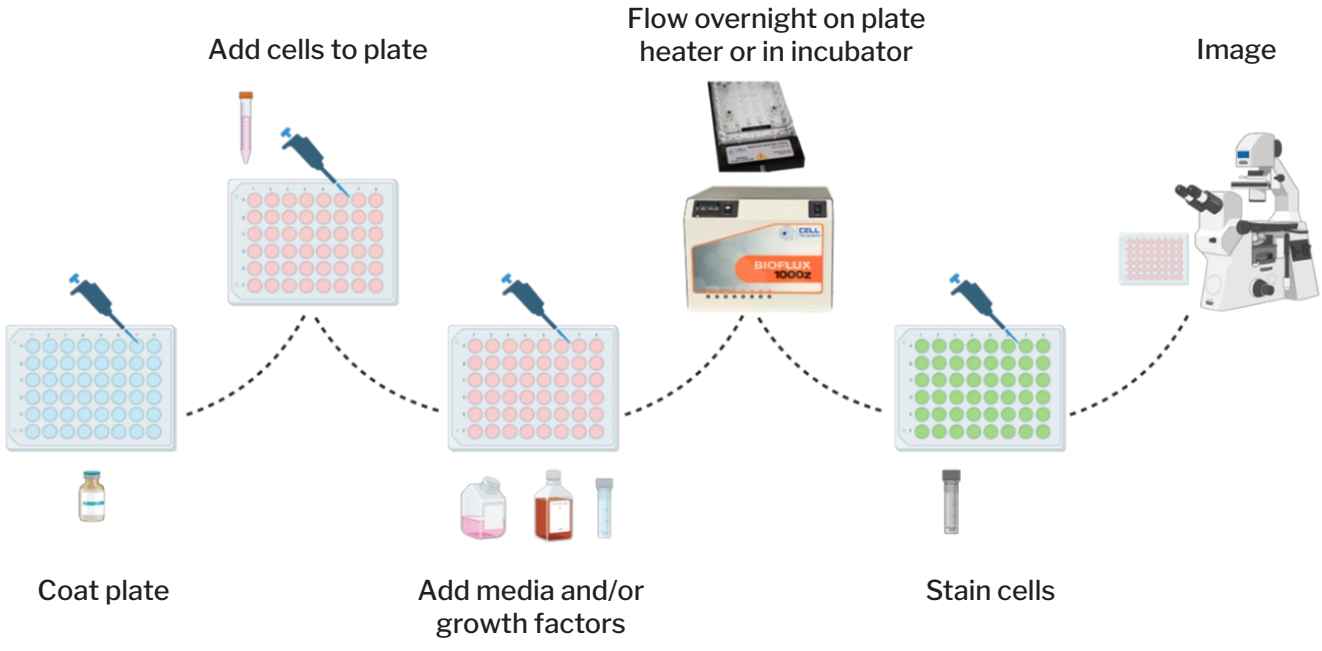
Stem cells have various uses, including disease modeling, personalized therapies, and drug discovery. Despite their utility, stem cells can be difficult to maintain and differentiate to the cell type of interest. The control of microenvironmental factors, including temperature, gas concentration, and the application of specific amounts of shear stress to undifferentiated stem cells enhances their expansion. Additionally, shear stress combined with growth factors has been shown to stimulate the differentiation of stem cells to specific cell types, such as endothelial cells and chondrocytes.

The BioFlux shear flow system enables the application of shear stress to adherent cells through accurate control of shear flow rate. Furthermore, BioFlux enables precise control of temperature and gas concentrations, making the system optimal for stem cell expansion and investigation. In this application note, we demonstrate how controlled shear stress can be used to differentiate mesenchymal stem cells into von Willebrand factor (vWF) expressing endothelial cells.

Key Highlights:

1) Differentiate mesenchymal stem cells to endothelial cells
2) Reduce stem cell differentiation time
3) Use various cell attachment substrates to differentiate stem cells

Stem Cell Workflow



Methods

Poly-L-lysine coating

Cryopreserved rat mesenchymal stem cells (Millipore) were propagated as directed by the manufacturer. 48-well BioFlux plates (Figure 1) were coated with poly-L-lysine at room temperature for 1 hour. The plates were then washed using spent media from stem cell cultures.

Stem Cell Differentiation

Cells were suspended in Mesenchymal stem cell expansion medium (Millipore) at a concentration of $5x10^6$ cells/mL and seeded into the microfluidic channels of the plate. Plates were incubated overnight under gravity flow. The following day, Mesenchymal stem cell expansion media was aspirated, and CO_2-independent media* (Invitrogen) supplemented with either 10% FBS, 2% FBS, or 2% FBS + 50 ng/mL recombinant rat vascular endothelial growth factor (VEGF) was added to the plate. The plates were placed on a BioFlux plate heater and media were perfused either by gravity flow or at 1 dyn/cm^2 for 48-100 hours.

Fibronectin and Matrigel coatings

To demonstrate the versatility of BioFlux plates, additional plates were coated with fibronectin or Matrigel (Corning) in a similar fashion as described above. Rat mesenchymal stem cells were seeded and grown as described in the previous section. Differentiated cells were stained with Hoechst 33342 and the contents of the endoplasmic reticulum, Golgi apparatus, and other membrane-bound organelles were labeled with wheat germ agglutinin.

Staining and Imaging

Flow was stopped to add media as necessary. Following perfusion, cells were fixed in the BioFlux plate using 1% paraformaldehyde for 30 minutes. Cells were then blocked and stained using a primary antibody (rabbit) against vWF (Abcam) and Alexa Fluor 594 phalloidin (Invitrogen). Following staining, cells were washed using PBS + 0.5% BSA for 10 minutes at 1 dyn/cm^2. Finally, Hoechst 33342 in PBS was added by gravity flow and images were captured using a Nikon TS100 microscope and a CCD camera (QICAM).

BioFlux plate

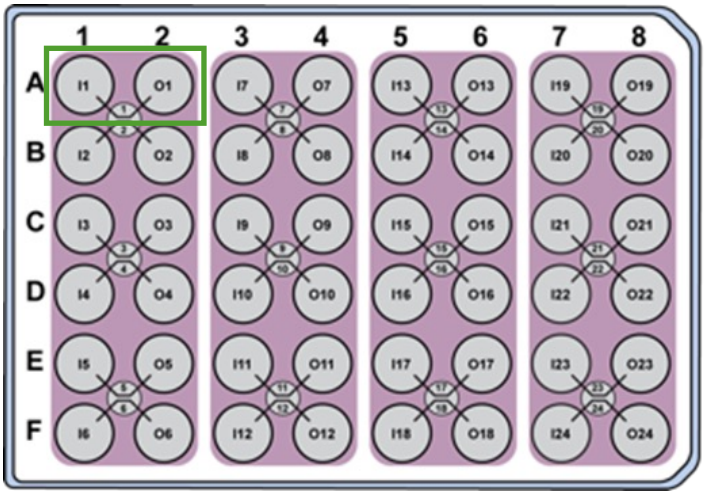


Figure 1. 48-well BioFlux plate. The green square highlights an inlet (A1) and outlet (A2) well with a viewing channel connecting the two wells. Each pair of inlet and outlet wells represent independent assays.

*5% CO_2 gas mixture used as the pressure source can be used as a substitute for CO_2-independent media

Results

The results of stem cell differentiation are presented in Figures 2 and 3. After 48 hours, all cells were differentiated, indicated by cytosolic expression of vWF. However, only stem cells cultured under shear flow with 10% FBS media were fully differentiated into endothelial cells after 48 hours. Interestingly, stem cells cultured under shear flow with 2% FBS showed similar vWF factor expression as stem cells cultured with 10% FBS without shear flow and greater vWF expression than stem cells cultured with VEGF and shear flow.

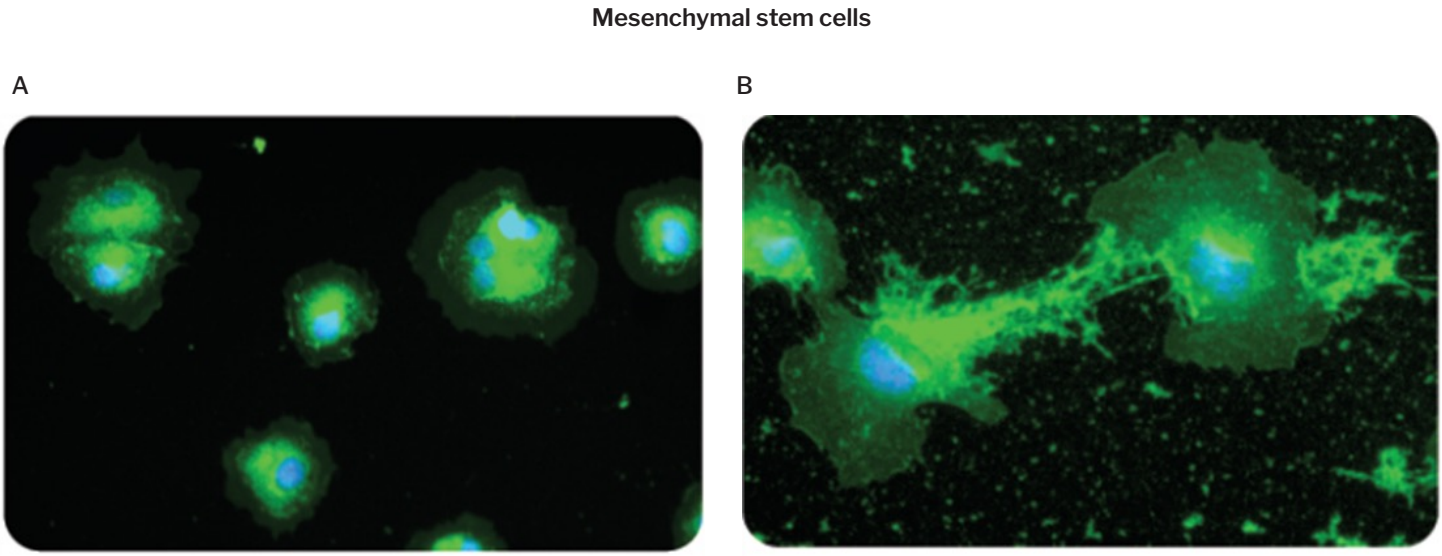


Figure 2. Rat mesenchymal stem cells grown under shear flow and stained with Hoerscht 33342 to identify nuclei (blue) and membrane-bound structures were labeled with wheat germ agglutinin (green). Cells were grown on a fibronectin coating (A). Cells shown during a division were grown on Matrigel (B).

von Willebrand expression

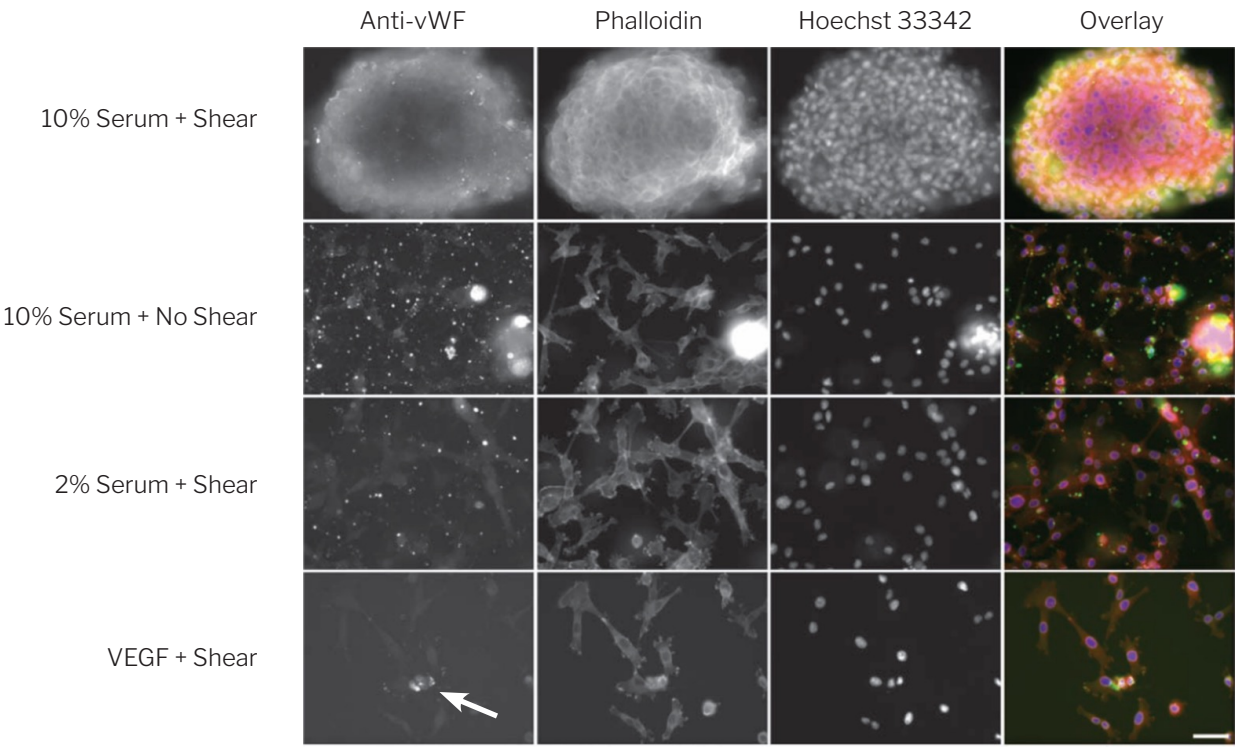


Figure 3. Micrographs of vWF expression in differentiating mesenchymal stem cells. Arrow indicates vWF expressing cells in the VEGF + shear flow condition. All fluorescence micrographs are after 48-hours of continuous flow or no flow, as indicated. Scale bar = 50 µm.

Discussion

Using the BioFlux shear flow system, we have demonstrated the rapid differentiation of mesenchymal stem cells to endothelial cells using shear flow. Together, with temperature control and growth factors, these data show that shear flow accelerates differentiation of stem cells to endothelial cells compared to either shear flow or growth factors alone, indicated by greater expression of vWF. These data demonstrate that the BioFlux shear flow system can be used to improve the efficiency of disease modeling, personalized therapies, and drug discovery by accelerating stem cell differentiation.

For more information on BioFlux Technology, visit cellmicrosystems.com/bioflux

CELL Microsystems®

801 Capitola Drive, Suite 10
Durham, NC 27713
info@cellmicrosystems.com