Spatially controlled stem cell differentiation via morphogen gradients: a comparison of static and dynamic microfluidic platforms

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The ability to harness the processes by which complex tissues arise during embryonic development would improve our ability to engineer complex tissue-like constructs *in vitro*—a long-standing goal of tissue engineering and regenerative medicine. In embryos, uniform populations of stem cells are exposed to spatial gradients of diffusible extracellular signaling proteins, known as morphogens. Varying levels of these signaling proteins induce stem cells to differentiate into distinct cell types at different positions along the gradient, thus creating spatially patterned tissues.

Here, we describe two straightforward and easy-to-adopt microfluidic strategies to expose human pluripotent stem cells (hPSCs) *in vitro* to spatial gradients of desired differentiation-inducing extracellular signals. Both approaches afford a high degree of control over the distribution of extracellular signals while preserving the viability of the cultured stem cells. The first microfluidic platform is commercially available and entails static culture, whereas the second microfluidic platform requires fabrication and dynamic fluid exchange. In each platform, we first computationally modeled the spatial distribution of differentiation-inducing extracellular signals. Then, we used each platform to expose hPSCs to a gradient of these signals (in this case, inducing a cell type known as the primitive streak), resulting in a regionalized culture with differentiated primitive streak (PS) cells predominately localized on one side and undifferentiated stem cells at the other side of the device. By combining this approach with a fluorescent reporter for differentiated cells and live-cell fluorescence imaging, we characterized the spatial and temporal dynamics of primitive streak differentiation within the induced signaling gradients. We envision that microfluidic approaches to create precisely controlled morphogen gradients will add to the stem cell and developmental biology toolkit, and may eventually pave the way to create increasingly spatially patterned tissue-like constructs *in vitro*.

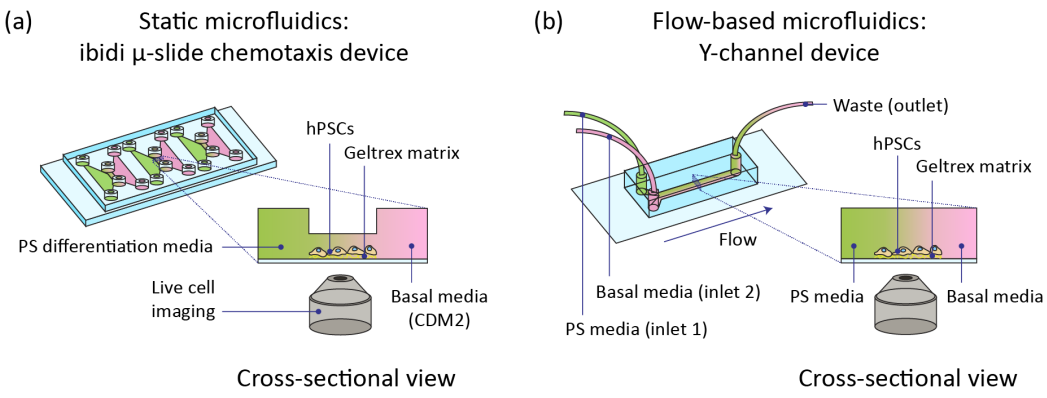

Figure 1: Schematic of microfluidic setups used to generate gradients of soluble signaling factors across MIXL1-GFP hPSCs. Cells undergoing differentiation into anterior primitive streak (PS) were observed with live-cell fluorescence microscopy. (a) Commercial static Ibidi µ-slide chemotaxis microfluidic device. (b) Flow-based Y-channel polydimethylsiloxane (PDMS) microfluidic device.

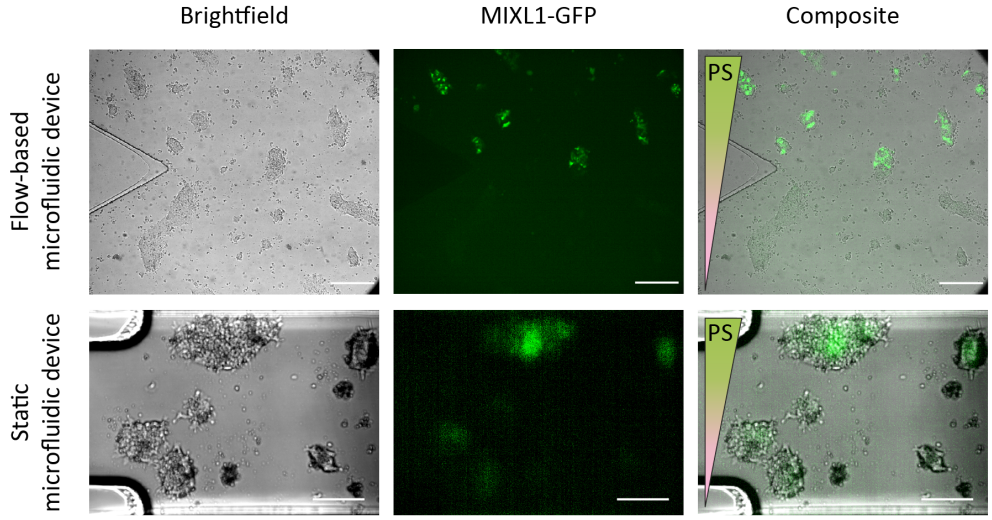

Figure 2: Brightfield and fluorescence micrographs of MIXL1-GFP hPSCs after 24 h of exposure to a gradient of anterior primitive streak (PS) factors in flow-based and static microfluidic devices. Fluorescent green cells (GFP-positive) indicate MIXL1 expression and thus the induction of primitive streak. Top row depicts cells in a flow-based Y-channel microfluidic device in the vicinity of the Y-junction. Flow rate at each inlet was 100 nL/min. Bottom row shows cells in a static commercial Ibidi microfluidic device. Scale bar, 250 µm.