IN VITRO CONSTRUCTION OF COMPLEX STEM CELL MICROENVIRONMENTS BY STENCIL-AND-FLIP CELL PATTERNING (SAF-CP)

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ABSTRACT

Embryonic development is a complex dynamic process where spatial organization of molecular signals direct stem cells into the 3 germ layers. Recapitulating this process *in vitro* will greatly facilitate the mechanistic understanding of embryonic development. However, current models are unable to reproduce the complex developmental environment adequately. To realize the aim of building a complex developmental model, we developed the Stencil-and-Flip Cell Patterning (SAF-CP) technique to present multiple spatially organized microenvironments to a single population of stem cells.

KEYWORDS: stem cells, development, cell patterning

INTRODUCTION

Embryonic development is a dynamic process and is controlled by molecular signals presented to stem cells by surrounding cells over space and time. To understand these processes, researchers have developed technologies that can mimic the *in vivo* environment in a culture dish, such as microcontact printing.[1-2] However, these systems only crudely reproduce the intricate *in vivo* developmental environment, where developing cells are in contact with multiple cell types and ECMs in a complex spatial arrangement.

To move closer to the goal of recapitulating embryonic development *in vitro*, here we demonstrate Stencil-and-Flip Cell Patterning (SAF-CP) to present multiple spatially organized microenvironments to a single population of stem cells. SAF-CP combines two established patterning technologies *i.e.,* stencil and Bio Flip Chip (BFC)[3] but uses them in a sequential aligned format to create complex *in vitro* microenvironments. We used the SAF-CP to spatially control the fate of stem cells within a single colony, demonstrating the utility of the SAF-CP in building *in vitro* models that can recapitulate embryonic development more realistically.

EXPERIMENTAL

Operations of SAF-CP

During the first step of SAF-CP, we use a PDMS stencil to generate dual microenvironments. Cell or ECM, such as gelatin, was patterned onto a substrate using the stencil (Fig 1A, i). The stencil was removed and the unpatterned substrate was backfilled with a second cell type or ECM (Fig 1A, ii). Stenciled substrates were incubated overnight before stem cell colonies were patterned with the BFC as previously

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described[3] (Fig 1A, iii). The assembly of the components during SAF-CP is shown in Fig 1B.

Cell culture

ABJ1 mouse embryonic stem cells (mESCs) were routinely maintained in self-renewing medium[3] while N2B27 serum-free differentiation medium was used to direct mESCs down the neuronal linage. STO feeder cells were cultured in DMEM, 10% FCS, 1% sodium pyruvate and 1% penicillin / streptomycin.

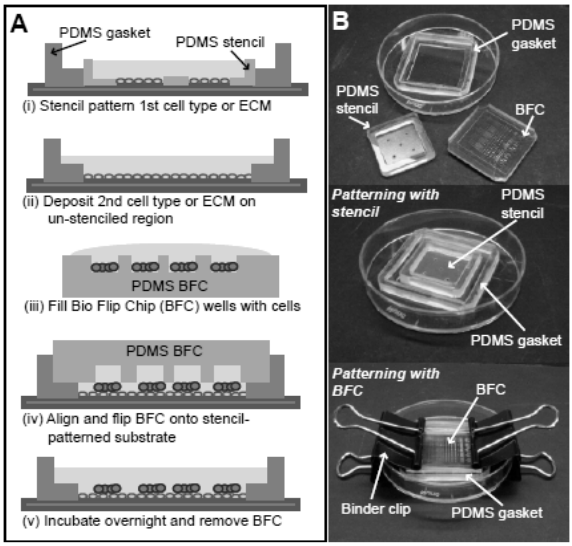

Figure 1. ***(A)*** *Schematic illustration of the Stencil-and-Flip Cell Patterning (SAF-CP) technique.* ***(B)*** *Components for performing the SAF-CP include a PDMS gasket, stencil and the Bio Flip Chip (BFC) (top panel). The middle and bottom panels show the assembly of the respective components during the stenciling and flipping steps of SAF-CP respectively.*

RESULTS AND DISCUSSION

The first step of SAF-CP is to generate dual microenvironments via stencil patterning. The dual microenvironment can be comprised of two different cells, cell & ECM, or two different ECMs. In the second step of SAF-CP, we use the previously developed BFC[3] to pattern cellular aggregates of different sizes onto the stenciled substrate. BFC cell patterning works by loading cells into wells in a PDMS chip (Fig. 1A, iii) and then "flipping" the PDMS chip onto a recipient substrate. The cells fall out of the wells and onto the second substrate while maintaining their alignment (Fig. 1A, iv). BFC has the advantageous feature that it can be used to pattern cells onto other cells or other ECM. In SAF-CP, we align the BFC wells with the stenciled micropatterns, allowing us to position the flipped cell population to span the dual stenciled microenvironments.

We used SAF-CP to selectively present differentiating and self-renewing microenvironments to single mESC colonies and investigate the spatial variation of the resulting mESC phenotypes. We first stenciled STO fibroblast populations (known to maintain mESC self-renewal) and deposited gelatin in the surrounding substrate (Fig. 2A). When cultured in serum-free differentiation media, mESC colonies on STO fibroblasts self-renewed due to factors produced by the STO cells (Fig 2A), while mESCs on gelatin differentiated down the neuronal lineage (Fig 2B). Next, we used SAF-CP to pattern mESC colonies spanning STO and gelatin micropatterns (Fig 2C). After 1 week of culture, the mESCs had a spatial distribution of self-

renewal (Oct4) and neuronal differentiation (Nestin) coinciding with the patterned microenvironments (Fig 2D).

CONCLUSIONS

This study demonstrates that we can use SAF-CP to spatially organize the local microenvironment and in turn direct the fate of stem cells within a single colony, bringing us closer to the goal of making *in vitro* embryos.

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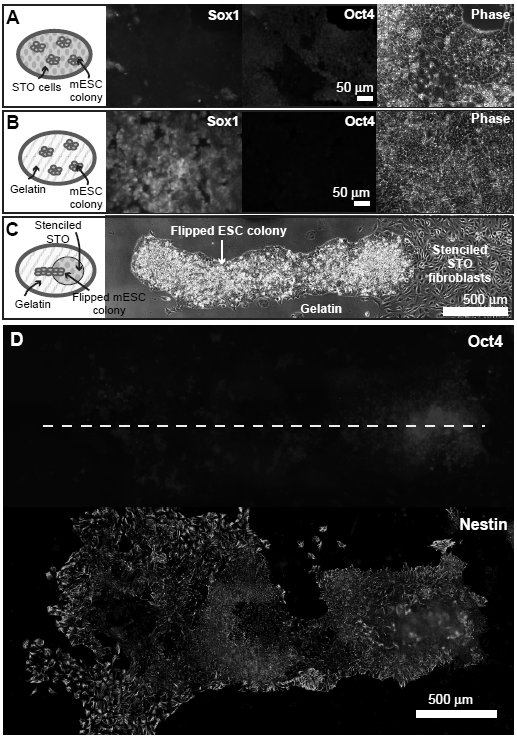

Figure 2. *Selective presentation of self-renewing and differentiating microenvironments to a single mESC colony using SAF-CP.* ***(A-B)*** *Self-renewing and differentiating microenvironments were maintained by (A) STO fibroblasts and (B) gelatin respectively in N2B27 medium as demonstrated by the expression of Sox1 (neuronal precursor marker) and Oct4 (self-renewal marker) after 7 days of culture.* ***(C)*** *A single 2000 x 200 μm mESC colony was flipped onto the interface of the STO and gelatin micropatterns.* ***(D)*** *The expression of Oct4 and Nestin (neuronal precursor marker) after 7 days of culture exhibited a spatial distribution coinciding with the patterned dual environments.*