Direct differentiation of human embryonic stem cells into neuronal lineage on nano line structures

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ABSTRACT

We introduce simple and direct differentiation of human embryonic stem cells (hESCs) on polymeric nanostructures.

KEYWORDS: Human embryonic stem cells(hESCs), Differentiation, Neuronal lineage, Capillay force lithography,nano line structures

INTRODUCTION

We introduce simple and direct differentiation of human embryonic stem cells (hESCs) on polymeric nanostructures. hESCs cultured on 350 nm polyurethane acrylate (PUA) line structures coated with 0.1% gelatin in DMEM/F12-SR were differentiated into neuronal lineage without additional regulator of differentiation such as retinoic acid or laminin. Previous researches reported that micro/nano structures induce stem cell differentiation into neuronal lineage with additional chemical cues [1-4]. However, direct differentiation of hESCs induced by nano-line features was not reported yet.

RESULTS AND DISCUSSION

Figure 1 shows a schematic diagram of the UV-assisted capillary force lithography (CFL). For fabricating PUA nano structures, an adhesion promoter was spin-coated on glass substrate followed by dropping of PUA resin. Subsequently, a nano-patterned or a flat PUA mold was placed on the PUA resin followed by curing by exposure to UV light.

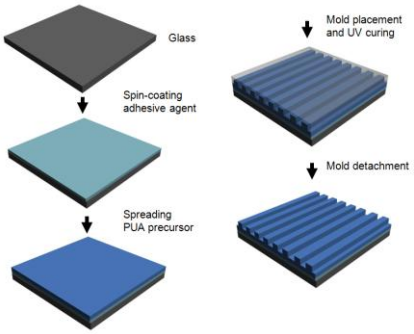

Figure 1. A schematic illustration for the fabrication of PUA nano structures by UV-assisted capillary force lithography (CFL)

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Figure 2a-b shows SEM images of PUA 350nm line structures (spacing of 350 nm, height of 500 nm) and flat substrate coated with 0.1 % gelatin. To enhance hESCs adhesion, PUA substrates were treated by oxygen plasma prior to 0.1% gelatin coating. hESCs were cultured in DMEM/FBS medium until hESCs were adhered on substrate and cultured in DMEM/F12-SR medium for five days. Figure 2c-d shows bright-field optical images of hESCs cultured on PUA 350nm line structures and flat surface for five days. hESCs cultured on PUA 350nm line structures showed contact guidance along the line direction. To measure cell morphology, hESCs were stained with anti-Tuj1(β-Tubulin Ⅲ) antibody, anti-GATA6(GATA binding protein 6) and DAPI.

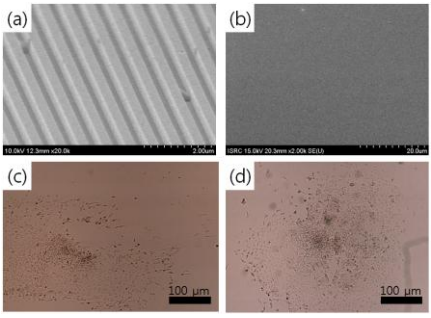

Figure 2. (a) SEM image of PUA 350nm line structures (spacing of 350 nm, height of 500 nm). (b) SEM image of PUA flat substrate. (c) Bright-field image of hESCs on 350nm PUA line structures. (d) Bright-field image of hESCs on PUA flat substrate.

Figure 3a and c show fluorescence images of immunostained hESCs cultured on PUA 350nm line structure for five days. Figure 3d is the bright-field image of Figure 3c. Figure 3b is the fluorescence image of immunostained hESCs cultured on PUA flat substrate. Mature neuronal marker Tuj-1 was detected in hESCs cultured on PUA 350nm line structure, whereas hESCs cultured on PUA flat surface showed endocrine pancreatic development marker GATA6.

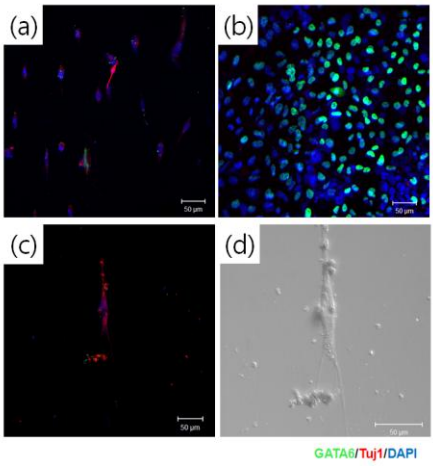

Figure 3. Immunofluorescent staining of GATA6, Tuj1 and DAPI. (a) hESCs on PUA 350nm line structures. (b) hESCs on PUA flat substrate. (c) Magnified view of (a). (d) Bright filed image of (c) showing hESCs on PUA 350nm line structure.

Figure 4 shows gene expression of undifferentiated hESCs, hESCs differentiated as embryoid bodies (EB), PUA 350nm line structures and PUA flat surface. The neuronal marker NeuroD1 was up-regulated on PUA 350nm line structures.

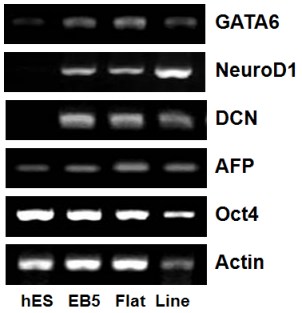

Figure 4. Gene expression analysis of undifferentiated hESCs, differentiated as embryoid bodies (EB), PUA 350nm line structures and PUA flat surface.

CONCLUSIONS

We introduced simple and direct differentiation of human embryonic stem cells (hESCs) on polymeric nanostructures. Our results showed that PUA 350nm line structures alone could induce hESCs into neuronal lineage. Our results can be applicable to designing a scaffold for neural tissue engineering.

ACKNOWLEDGEMENTS

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