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Nanogrooved substrate promotes direct lineage reprogramming of fibroblasts to functional induced dopaminergic neurons

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ABSTRACT

The generation of dopaminergic (DA) neurons via direct lineage reprogramming can potentially provide a novel therapeutic platform for the study and treatment of Parkinson's disease. Here, we showed that nanoscale biophysical stimulation can promote the direct lineage reprogramming of somatic fibroblasts to induced DA (iDA) neurons. Fibroblasts that were cultured on flat, microgrooved, and nanogrooved substrates responded differently to the patterned substrates in terms of cell alignment. Subsequently, the DA marker expressions, acquisition of mature DA neuronal phenotypes, and the conversion efficiency were enhanced mostly on the nanogrooved substrate. These results may be attributed to specific histone modifications and transcriptional changes associated with mesenchymal-to-epithelial transition. Taken together, these results suggest that the nanopatterned substrate can serve as an efficient stimulant for direct lineage reprogramming to iDA neurons, and its effectiveness confirms that substrate nanotopography plays a critical role in the cell fate changes during direct lineage reprogramming.

1. Introduction

The unrestricted conversion of cell fate, which was first noted by the generation of induced pluripotent cells (iPSCs) via epigenetic reprogramming [1], provides critical evidence for the plasticity of the somatic epigenome. Based on this concept, numerous studies have demonstrated the feasibility of direct lineage reprogramming from one somatic cell type into another, including cardiomyocytes [2] and neurons [3,4]. A pioneering study on neuronal lineage reprogramming revealed that the ectopic expression of Ascl1, Brn2, and Myt1l (ABM) induces the direct conversion of mouse embryonic fibroblasts (MEFs) and postnatal fibroblasts into functional neurons [3]. Furthermore, we and others have also demonstrated that somatic fibroblasts can be directly reprogrammed to neuronal subtypes such as dopaminergic (DA) neurons [5–8], by the overexpression of Ascl1, Pitx3, Nurr1 and Lmx1a (APNL) transcription factors [6]. The generation of induced DA (iDA) neurons from direct lineage reprogramming has several advantages over the iPSC technology. Because the desired cells generated via direct conversion do not pass through and re-differentiate from a pluripotent state, direct conversion enables the production of patient-specific cells from abundant cell sources (mostly fibroblasts), and can provide solutions to safety problems (i.e., teratoma formation) and economical aspects (i.e., saving time and cost) for further clinical applications. Nevertheless, the low efficiency of direct conversion (<10% of plated cells) [2,4,6] remains a significant barrier to reprogrammed cell-based transplantation therapy. Thus, several studies have endeavored to enhance the conversion efficiency, but the approaches have been constrained within biochemical stimulations [5,6].

Cellular behaviors can be modulated not only by biochemical signals but also by biophysical cues, especially from the extracellular matrices (ECMs). ECMs contain a complex set of information (e.g., mechanical properties and topography) that can trigger complex cellular responses [9]. Specifically, the microscale and nanoscale substrate topography of ECMs or cell-culture substrates is one of the most significant factors that can regulate cell adhesion, proliferation, migration, and differentiation [9,10]. Regarding the

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cellular fate control via the sub-microscale interactions between cells and biomaterial substrates, previous studies have reported that nanopatterned substrates, specifically those with ridge/groove patterns, can promote the commitment of human embryonic stem cells [11], human iPSCs [12], and human mesenchymal stem cells [13] to the neuronal lineage. In addition to the alteration of lineage commitment during the stem cell differentiation process, a recent report demonstrated that submicroscale topography can expedite the nuclear reprogramming process of somatic fibroblasts (MEFs) through epigenetic state regulation [14]. Namely, topographical cues could be one of the most significant determinants of engineering cellular fate, and their impact is comparable to that of biochemical factors, to transcend the limits of epigenetic states. Furthermore, nanoscale topography may provide an environment more conducible for cells to be transformed into neurons than the microscale counterpart, based on the preferred neuronal differentiation of various types of stem cells on nanopatterned substrates [11–13].

In this study, we demonstrated that nanoscale topography can promote the direct conversion of MEFs into functional iDA neurons. Because topographical cues could promote epigenetic changes [14], we expected that nanotopographical cues combined with ectopic gene expression could effectively promote MEFs to convert their phenotypic profiles into those of DA neurons. The possible mediators of the conspicuous epigenetic changes were also examined, especially focusing on mesenchymal-to-epithelial transition (MET) and histone modification. We attempted to demonstrate that nanotopographical stimulation has a synergistic effect on the direct lineage reprogramming via the ectopic expression of transcription factors, Ascl1, Pitx3, Nurr1 and Lmx1a (APNL), which could effectively help MEFs to be converted into DA neurons, one of the major neuronal subtypes (Fig. 1).

2. Results

Preparation of microgrooved and nanogrooved substrates. Using ultraviolet (UV)-assisted capillary force lithography, two types of patterns (microgrooved and nanogrooved substrates) were fabricated by changing the groove width (1.2 μm and 400 nm, respectively), while the ridge size remained the same (300 nm). Scanning electron microscope (SEM) images confirmed that the intended patterns were replicated with a good fidelity, without any local changes in the dimensions of the ridges or grooves (Fig. 2A).

Nanogrooved substrate induces cell alignment. Prior to examining whether microscale or nanoscale substrate topography influenced cell fate during the direct lineage reprogramming of

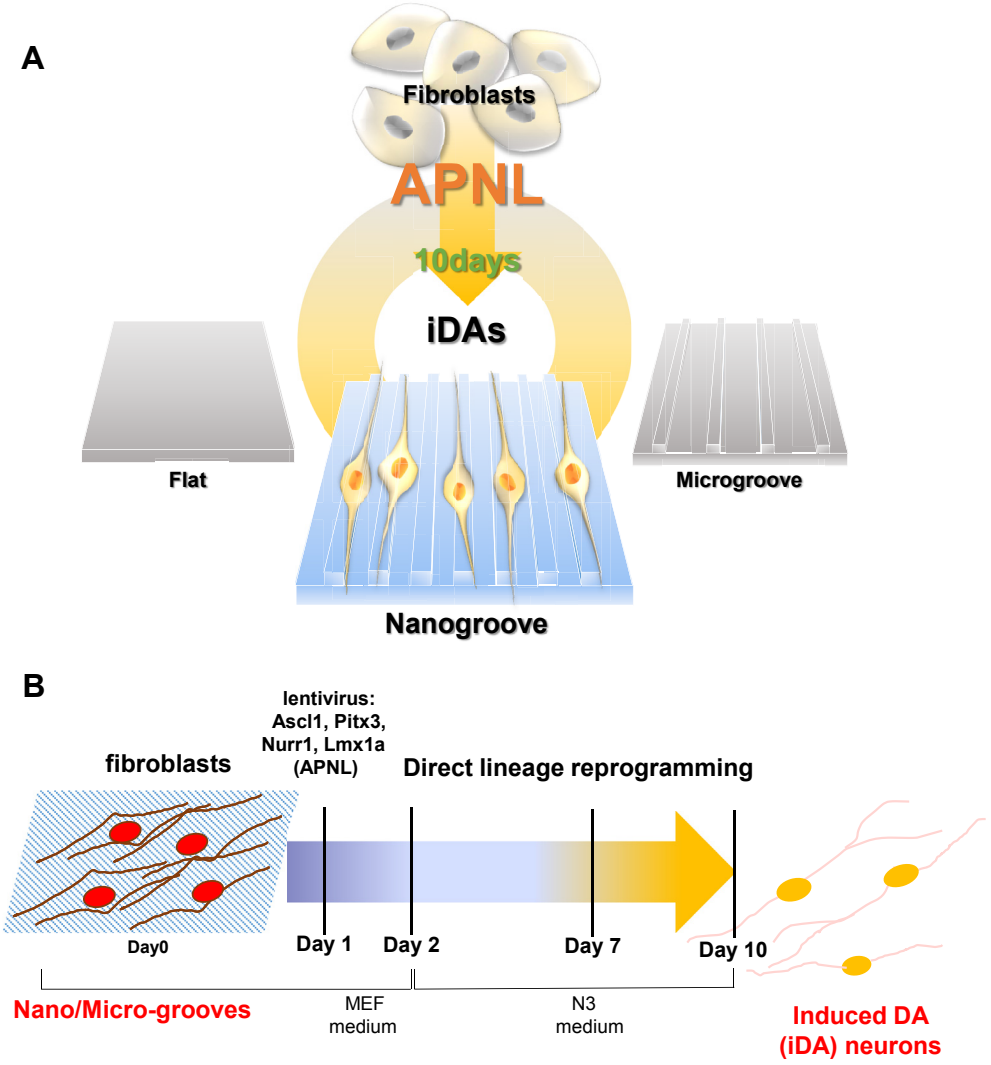

Fig. 1. Generation of iDA neurons on flat, microgrooved, and nanogrooved substrates. Schematic diagrams describing (A) the concept of this study and (B) the direct conversion protocol.