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Microlens topography combined with vascular endothelial growth factor induces endothelial differentiation of human mesenchymal stem cells into vasculogenic progenitors

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ABSTRACT

Cell therapy for vascular damage has been showing promises as alternative therapy for endothelial dysfunctions since the discovery of the endothelial progenitor cells (EPCs). However, isolated EPCs from peripheral blood yield low cell amounts and alternative cell source must be explored.

The aim of this study was to investigate the influence of topography on the endothelial differentiation of an alternative cell source – human mesenchymal stem cells (hMSCs) from bone marrow. Utilizing the MultiARChitecture (MARC) chip, a systematic screening of variety of patterned surfaces and different medium compositions was performed. While topographical patterns alone induce endothelial differentiation, a synergistic enhancement was observed when topography was combined with a medium enriched with vascular endothelial growth factor (VEGF). The 1.8 μm diameter convex microlens pattern in combination with the VEGF enriched medium was shown to be the most efficient on the endothelial differentiation, yielding up to 10% of $CD34^{+}CD133^{+}KDR^{+}$ marker expressing differentiated hMSCs as analyzed by flow cytometry. The quantified tube-like structures in the Matrigel assay *in vitro* indicated a vasculogenic potential of these endothelial progenitor-like differentiated hMSCs that was investigated further in a Matrigel plug assay *in vivo* in a rat for seven days. Explanted Matrigel plugs were processed with hematoxylin-eosin (H&E) and anti-Ulex Europaeus agglutinin (UEA-1) staining to visualize the capillaries and to identify the presence of human cells. The hMSCs cultured on the 1.8 μm diameter convex microlens in a medium enriched with VEGF, implanted in a Matrigel plug in a rat, showed the highest capillary density, the highest $UEA\text{-}1^{+}$ capillary density, as well as the highest $UEA\text{-}1^{+}$ cell survival density that were not included in the vasculogenesis. These findings indicate the active participation of the vasculogenic hMSCs in the vasculogenesis. The endothelial differentiation of hMSCs using this synergistic combination of microlens and VEGF enriched medium was also demonstrated in hMSCs from different male and female donors. The culture platform with combination of topography and biochemical cues could generate vasculogenic cell populations that may prove useful in vascular damage or other clinical applications.

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1. Introduction

Cardiovascular diseases (CVD) are the leading cause of death worldwide [1], with endothelial dysfunction as the major cause [2–6]. There are limited therapeutic options for endothelial dysfunction and both pharmacological and surgical approaches do not fully improve endothelial function or support endothelial regeneration [7].

Much interest has been attracted in exploring the potential of different therapy options for vascular diseases. Endothelial progenitor cells (EPCs) have been hypothesized to be a potential cell therapy for vascular repair [8]. Although EPCs are highly proliferative and capable of differentiating into mature endothelial cells, their use is limited by difficulties in the isolation of sufficiently large numbers of EPCs required for the desired therapeutic effect [9]. The derivation of vascular healing cells from an alternative source is

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therefore in demand. Bone marrow mesenchymal stem cells (MSCs) are a potential alternative source of cells, with favorable properties such as high efficiency in expansion, low immunomodulation effect and high differentiation potential [10]. In this study, we hypothesized that the biophysical environment could induce the differentiation of human MSCs (hMSCs) into cells with vasculogenic properties. We also hypothesized that a synergistic effect of a topographical cue and biochemical cue will enhance the endothelial differentiation of hMSCs. By performing a systematic screening, an optimal combination of biochemical and topographical cue could be identified, leading to an enhanced generation of vasculogenic cells with a potential for cell therapy applications.

Cell therapy is an emerging approach for attenuation of endothelial dysfunction and could potentially enable full vascular regeneration [11]. A variety of cell types have been studied in endothelial function improvement and regeneration, with adult stem cells and pluripotent stem cells as the two major groups studied [11,12]. The MSCs, as an adult stem cell source, have been shown to differentiate into endothelial-like cells given specific biochemical cues such as vascular endothelial growth factor (VEGF) [13]. In addition, MSCs have also been studied for their interaction with their physical environment. High throughput platform screening with a number of topographies revealed that physical cues can induce MSC differentiation into osteocytes [14,15] or even differentiation into neuronal cell types [16,17]. Although a number of elegant studies showed the influence of mechanical stimuli (shear stress, stretch, translational and rotational strain) on vascular endothelial cells and mesenchymal stem cells, there has been very limited research on how topographical cues influence endothelial differentiation of hMSCs [18–24]. Hence, a systematic evaluation of topographical cues could lead to identification of an efficient pattern stimulus for endothelial differentiation.

The following study represented a systematic screening of a variety of topographical cues on the MultiARChitecture chip (MARC chip), demonstrating for the first time the influence of topographical cues alone on the endothelial differentiation of hMSCs. The screening of topographical cues was combined with defined culture media with different composition to identify the optimal combination of physical and biochemical cues that would enhance the endothelial differentiation of hMSCs into endothelial progenitor-like cells with vasculogenic properties. The differentiated hMSCs were characterized *in vitro* by their cell marker expression and tube formation capability in Matrigel. Moreover, the vasculogenic capability of the differentiated hMSCs were demonstrated in a Matrigel plug *in vivo* assay, which showed the potential for applications in cell therapies for the treatment of endothelial dysfunction in human and veterinary medicine.

2. Experimental section

2.1. Fabrication of Multi-ARChitecture (MARC) chip master and MARC chips

The MARC chip master was fabricated as previously described [25,26]. Briefly, various topographies (Topography 1-16 and unpatterned control, Table 1A) were imprinted on polycarbonate (PC) via nanoimprint lithography (NIL). The chosen patterns were cut into 2 mm × 2 mm pieces, which were then assembled on a silicon substrate, and fused into a single coherent layer using polydimethylsiloxane (PDMS, Sylgard 184, Corning) as a binding material, leaving an unpatterned border around the topographies. The MARC chip master was surface-treated with perfluorodecyltrichlorosilane (silane). PDMS (3g) in a 10:1 ratio of elastomer to crosslinking agent was used for replication of the MARC chip (Schematic diagram S1A). The MARC chip was cured overnight (approximately 12 h) at 60 °C and demolded at room temperature.

Table 1
Table of patterns and medium compositions. (A) List of patterns on the MultiARChitecture chip (MARC chip) and patterns replicated in the MARC chip replicas. Pattern number, isotropy and size are indicated. (B) List of media labels. The medium compositions are indicated.

Table 1A

Description	Size	Topography on MARC chip	Topography number	Selected topography
Anisotropic	Nano	250 nm lines, 250 nm space, 110 nm height	2	
		250 nm lines, 250 nm space, 250 nm height	1	
		70 nm lines, 460 nm space, 40 nm height	3	
	Micro	1 μm lines, 2 μm space, 80 nm height	6	Gratings
		2 μm lines, 1 μm space, 120 nm height	5	
		2 μm lines, 2 μm space, 2 μm height	4	
		2 μm lines with perpendicular 250 nm lines in the groove	7	
		2 μm line with parallel 250 nm lines in the groove	8	
		2 μm lines with 250 nm wells in the groove	9	
Isotropic	Nano	250 nm wells, 400 nm pitch, 250 nm depth	16	
	Micro	1 μm diameter, 1 μm pitch, 0.3 μm sag convex microlens	10	
		1 μm diameter, 1 μm pitch, 0.3 μm sag concave microlens	11	
		1 μm pillars, 6.5 μm pitch, 1 μm height	15	
		1.8 μm diameter, 2 μm pitch, 0.7 μm sag convex microlens	12	Convex microlens
		1.8 μm diameter, 2 μm pitch, 0.7 μm sag concave microlens	13	
		2 μm wells, 12 μm pitch, 2 μm depth	14	Wells

Table 1B

Medium compositions and labels

Medium label	Medium	Medium compositions
GM	Mesenchymal stem cells growth medium	Mesenchymal cell growth supplement, L-Glutamine, Gentamycin/Amphotericin
GM + V	Mesenchymal stem cells growth medium supplemented with vascular endothelial growth factor	Mesenchymal cell growth supplement, L-Glutamine, Gentamycin/Amphotericin, Vascular endothelial growth factor
LG + E	Endothelial differentiation medium without vascular endothelial growth factor	Low glucose Dulbecco's modified Eagle's medium supplemented with fetal bovine serum, hydrocortisone, Penicillin/Streptomycin, Epidermal growth factor
EDM	Endothelial differentiation medium	Low glucose Dulbecco's modified Eagle's medium supplemented with fetal bovine serum, hydrocortisone, Penicillin/Streptomycin, Epidermal growth factor, Vascular endothelial growth factor

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