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초대배양된 인체 족세포의 분화를 위한 microfluidic 시스템 개발

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A Novel in Vitro Protocol using Microfluidic System for Human Primary Podocyte Differentiation Induction

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Podocyte de-differentiation is one of major problems among many causes of glomerular diseases. Recently, intensive researches on the topic is emerging, yet lengthy podocyte differentiation process hinders a progress. Kruppel-like factor 15 (KLF 15) was reported to be a novel transcriptional regulator of podocyte differentiation and its expression was increased by retinoic acid (RA) which reduced the differentiation time of podocytes. But, the duration of podocyte differentiation process remains long. Here, we present a novel in vitro protocol to induce podocyte differentiation. To mimic in vivo biological environment of glomerulus, a polydimethly siloxane (PDMS) microfluidic device was used. Microfluidic devices have been used to simulate the activities, mechanics and physiological response of various organs such as lung, liver, and etc. The human primary podocytes were cultured in the 500 μm by 130 μm microfluidic channel and were stimulated with a laminar fluidic shear stress of 0.5 dyne/cm^2 for 5 days. Various dose of RA was used to promote podocyte differentiation. In this research, we were able to reduce the time required for podocyte differention with higher shear stress and higher dose of RA. A couple of tests was conducted to verify the podocyte differentiation through the protocol. First, the phenotype of podocytes changed from cobblestone like shape to arborized cells. Through immunofluorescence staining, the increased synaptopodin and ZO-1 expression was confirmed. Moreover, KLF 15 expression was also increased. These molecules were also quantified with mRNA expression through qPCR. The size of podocytes became larger and the height of podocytes increased by a factor of twofold. Lastly, the podocytes aligned with the direction of flow. Through this research, we were able to promote podocyte differentiation in the shorter processing time with microfluidics and RA. Yet, more effort to mimic biological aspects of kidney such as complex structure with multiple cell layers and round surface is necessary.

Key Words: 족세포 분화, 미세유체공학, KLF-15
Podocyte differentiation, Microfluidics, KLF-15