論 文 の 内 容 の 要 旨

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論 文 題 目

Recovery of Cryo-injured Rabbit Urethras by Biofabricated C-shaped Adipose-derived Mesenchymal Cell Structures

（ウサギ尿道凍結傷害モデルを用いたC型脂肪由来間葉系細胞構造体による尿道再生の試み）

（論文の内容の要旨）

【Background and Purpose】 Urethral tissue damage can cause stress urinary incontinence or urinary retention, and there are very few long-term, effective treatments. In our previous study, we have shown that direct injection of single autologous adipose-derived mesenchymal stem cells (AMCs) into cryo-injured urethra of rabbits restored structures and function. However, there was the major hindrance with the direct injection of cells having low survival, retention and integration of transplanted cells. Therefore, for structural and functional recovery of urethral tissues, we designed and constructed C-shaped AMCs by using three-dimensional (3D) bioprinter system, which could be transplanted in damaged urethras without obstructing the lumen.

【Material and Methods】 Adipose tissues harvested from 10-weeks old female New Zealand White rabbits were cultured and labeled with a fluorescent cell linker. After the culture, the adherent proliferating cells were seeded into 96 well plates to form cell-aggregation called spheroid. All spheroids were positioned in 3D structure according to a predesigned configuration to form C-shape by a robotic biofabrication system. The biofabricated structures were perfusion cultured for 7 days. Exposed urethra was sprayed with liquid nitrogen for 20 seconds, and small incision within the cryo-injured region was made. Following, the structure was immediately transplanted into the incision (n=4). As control, sham surgeries without the structure were performed (n=4). Two and four weeks after transplantation, the urethras were harvested for histological and immunohistological analysis.

【Results】 Before transplantation, the C-shaped AMC structures expressed the mesenchymal cell marker STRO1, and extracellular matrices; integrin and cadherin. In the gross anatomy, sham-operated urethra seemed narrowed and diverted. However, AMC structure transplanted urethra seemed wider and straight. Histological investigations showed that there was presence of distinct and regenerated muscle structures at 4 weeks after transplantation. The cells within the transplanted structures differentiated into skeletal, or smooth muscle cells and formed layered muscle structures at the surrounding neighborhood of transplanted regions at 4 weeks after transplantation. Also, the cells within the structures were positive for nerve cell markers. The cells within the structures secreted growth factors; vascular endothelial growth factor and nerve growth factor, and cytokines; transforming growth factor-β1 and tumor necrosis factor-α. At two and four weeks after sham surgery in which no biofabricated structure was transplanted, the urethral wound site contained collagen fibers in the extracellular matrix along with damaged skeletal and smooth muscle layers. However, in the transplanted urethral tissues, the collagen fibers were located between the recovering skeletal and smooth muscle layers. The transplanted urethras decreased P4HB- and HIF1α-positive cells, and apoptotic cells compared to the control urethras.

【Conclusions】 The biofabricated C-shaped AMC structures might have potential to regenerate the urethra by differentiating into skeletal muscle cells, smooth muscle cells, and nerve cells. Furthermore, these cells secreted growth factors and/or cytokines that likely enhanced myogenesis and neurogenesis. The transplanted urethra showed that development of urethral strictures during the recovery process was inhibited. Therefore, the transplantation of C-shaped biofabricated AMC structures has the potential to provide novel and effective treatments for storage or voiding dysfunctions associated with damaged urethras.