論 文 審 査 の 結 果 の 要 旨

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（論文審査の結果の要旨）

Stress urinary incontinence is an involuntary leakage of urine through urethra during physical activity that causes an increase in abdominal pressure, and there are few long-term, effective treatments.

We designed and constructed C-shaped structures composed of adipose-derived mesenchymal cells (AMCs) by using three-dimensional (3D) bioprinter system, which could be transplanted in damaged urethras without obstructing the lumen for the structural and functional recovery of the urethral tissues. Adipose tissues harvested from rabbits were cultured and labeled with a fluorescent cell linker, PKH26. After the labeling, the adherent proliferating cells were seeded in 96-well plates to make spheroids. The formed spheroids were assembled according to predesigned configuration by robotic biofabrication system. The biofabricated structures were perfusion cultured for 7 days to attain the desired structural organization and robustness.

Exposed urethra was sprayed with liquid nitrogen for 20 seconds, and a small incision within the cryo-injured region was made. Following the biofabricated structure was immediately transplanted within the incision and the incision was closed. As controls, sham operations without the structure was performed. Finally, two and four weeks after operation, the urethras were harvested for histological and immunohistological analyses.

Gautam Silwal Sudha showed results of these investigations, as below.

1. In gross anatomy, sham-operated urethra seemed narrowed and diverted. However, the structure-transplanted urethra was patent.
2. At 4 weeks after transplantation, cells within the transplanted structure differentiated into skeletal muscle cells, smooth muscle cells or nerve cells. These differentiated cells formed distinct muscle layers at the surrounding neighborhood of the transplanted structures.
3. The AMCs within the transplanted structures secreted higher growth factors and cytokines comparing to sham-operated control urethras.
4. Within the control urethras, collagen fibers invaded the spaces of the damaged skeletal and smooth muscle layers. In contrast, in the structure-transplanted urethra, collagen fibers were located between the recovering skeletal and smooth muscle layers.
5. The transplanted urethra decreased P4HB-, HIF1-α positive or apoptotic cells comparing to sham controlled urethra.

These results suggested that the biofabricated C-shaped structures might regenerate urethra by differentiating into muscles and nerve cells, and secretion of growth factors and cytokines that likely enhanced myogenesis and neurogenesis. Therefore, the transplantation of biofabricated C-shaped AMC structures has potentials to be effective treatments for stress urinary incontinence associated with damaged urethras.

以上の研究結果から主査、副査は一致して本論文を学位論文として価値があるものと認めた。