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Imamura T[1], Ishizuka O[1], Ogawa T[1], Yokoyama H[1], Minagawa T[1], Nakazawa M[1], Nishizawa O[1]
1. *Shinshu University School of Medicine*

TRANSPLANTATION OF CELL SHEETS PRODUCED FROM BONE MARROW-DERIVED CELLS RECONSTRUCTS FUNCTIONAL URINARY BLADDERS IN RADIATION INJURY RAT MODELS

Hypothesis / aims of study

Direct injection of bone marrow-derived cells into radiation-injured rat urinary bladders could reconstruct functional bladder tissues. (1) The direct injection methods need to use single cells. The cultured cells form monolayers with extracellular matrices (ECM) that supports cell adhesion and cell-to-cell communication. However, the single cells are prepared by destroying the monolayers with enzymatic treatments. Cell sheet-based tissue engineering by using temperature-sensitive culture dishes can provide a monolayer cell sheet with full preservation of the cell-cell contacts and ECM. In this study, we determined if bone marrow-derived cell sheets could reconstruct functional urinary bladders.

Study design, materials and methods

Twenty female 10-weeks Sprague-Dawley (SD) rats were anesthetized, and then the pelvic region containing the urinary bladder, which was 1-cm diameter circle bordering on the pubic bone was irradiated with 2Gy. The irradiation was performed once a week for 5 weeks. Following the last radiation exposure, the rats were maintained for 2 weeks. (1) Bone marrow cells were harvested from both femurs of two male 17-weeks Tg-SD (GFP) rats. The cells were cultured for 7 days, and then the adherent proliferating cells were transferred into temperature-sensitive culture dishes ($0.5x10^6$ cells/dish; CellSeed Inc., Tokyo, Japan). The transferred bone marrow-derived cells were culture for 2 days. Two days after, by lowering temperature below at 32˚C, the cultured cells were harvested as a monolayer cell sheet. The cell sheet was patched (transplanted) on the irradiated bladder wall (n=10). The control rats were similarly operated with cell-free sheet (n=10). At 4 weeks after transplantation, cystometric and histological investigations were performed.

Results

At 4 weeks after transplantation, the threshold pressure (17.59±2.56 cmH_2O), voiding interval (6.83±0.75 min), micturition volume (1.25±0.12 ml), and bladder capacity (1.27±0.12 ml) of the cell sheet-transplanted rats were significantly higher than these of the cell-free control rats (10.93±0.69 cmH_2O, 4.30±0.61 min, 0.73±0.12 ml, 0.78±0.12 ml; $P<0.05$, respectively), while the basal and micturition pressure did not show any differences (Figure A and B). In addition, residual volume of the cell sheet-transplanted rats (0.01±0.01 ml) was significantly lower compared to the control rats (0.05±0.03 ml; $P<0.05$). The proportion of smooth muscle layers (0.28±0.02) and acetylcholine esterase-positive cells (0.04±0.01) in the cell sheet-transplantation group were significantly higher than these in the control group (0.18±0.01, 0.01±0.004; $P<0.01$, respectively, Figure C-F). The number of the apoptosis cells in the cell sheet group (4.46±0.49 cells) was significantly lower than that in the control group (13.74±1.77 cells; $P<0.01$, Figure G and H).

Interpretation of results

Previously, we reported that the radiation-injured urinary bladders exhibited shorter voiding interval, lower micturition volume and bladder capacity, and larger residual volume compared to the intact urinary bladders. (1) The cell-free control rats maintained the urinary dysfunctions. In contrast, the bone marrow-derived cell sheet-transplanted rats improved bladder functions. In addition, the cell sheet-transplanted rats showed the reconstruction of bladder tissues.

Concluding message

This study showed that the transplantation of bone marrow-derived cell sheet into the radiation-injured urinary bladders reconstructed functional bladders. Therefore, the cell sheet-based tissue engineering had great potentials to develop the regenerative medicine of lower urinary tracts.

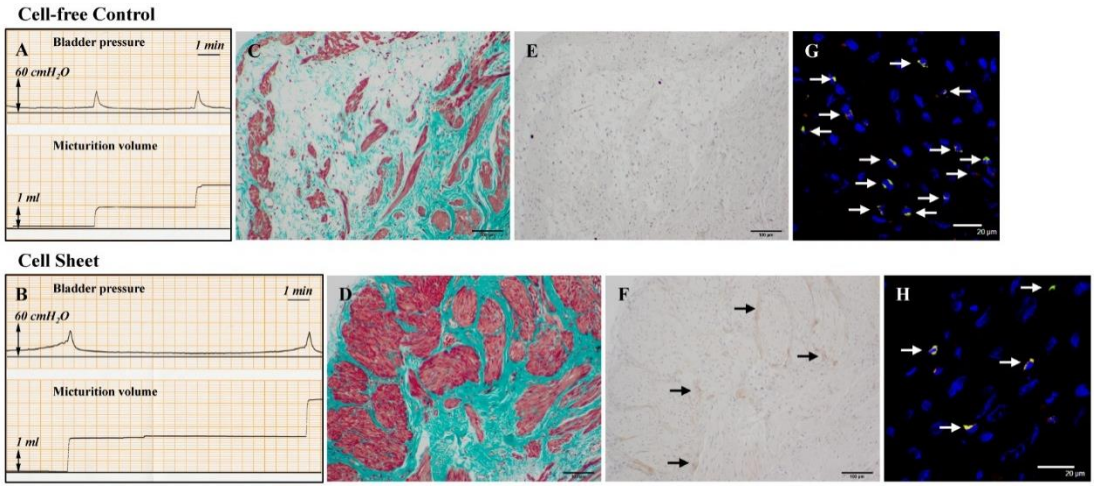

Figure. Effects of cell sheet transplantation. Top: cell-free control group, Bottom: cell sheet-transplantation group. A and B: typical micturition patterns, C and D: masson trichrome stain, E and F: acetylcholine esterase stain (arrows: positive cells), G and H: detection of apoptosis (arrows: apoptosis cells).

References
1. Imamura, et al. Tissue Eng. Part A 18: 1698-1709, 2012.

Disclosures
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