Experimental study of regeneration of intervertebral disc after allograft of cultured annulus fibrosus cells using tissue engineering method


Introduction: Annulus fibrosus (AF) is histologically categorized as fibrocartilage, but it is the largest avascular tissue in the human body, and its capacity for tissue regeneration is as low as that of articular cartilage. In cases where nucleotomy is performed as a surgical treatment for intervertebral disc herniation, there is little regeneration of tissue, and intervertebral disc degeneration is inevitable. The ideal surgical treatment for intervertebral disc herniation would consist of nucleotomy as non-invasively as possible, followed by filling of the lacuna generated by surgery with intervertebral disc tissue, resulting in morphological, histological, and functional regeneration. For this study, we improved on the collagen/keratin-combined scaffold currently being sold as a cell culture carrier, creating our own transplantation carrier atelocollagen honeycomb-shaped scaffold with a membrane seal. We allografted three-dimensional cultured AF cells in this high-density, into the lacunae of intervertebral discs whose nucleus pulposus (NP) had been vaporized with an ICG-dye-enhanced laser[1]. The regeneration of the intervertebral disc was studied from the viewpoint of the viability and the histological status of the allografted cultured AF cells.

Materials and Methods: Three-dimensional culture and PKH-26 labeling: AF from the intervertebral discs of 20 female Japanese white rabbits (4 weeks old; average weight, 1 kg) was digested in F12/DMEM containing Pronase and bacterial Collagenase to isolate AF cells. The isolated cells were then labeled with PKH-26 fluorescent dye[2] and seeded in atelocollagen honeycomb-shaped scaffolds with a membrane seal, in 48 specially prepared well-plates, at a density of 2x10^6 cells per scaffold carrier. PKH-26 fluorescence does not transfer to other cells, but does transfer to daughter cells. The cells were then cultured in F12/DMEM supplemented with 10% fetal bovine serum, 50µg/ml ascorbic acid, and 100U/ml streptomycin-penicillin at 37°C in an atmosphere of 5% CO2 and 95% air with anti-BrdU-fluorescin-F(ab')2 fragments of monoclonal antibody[3]. This method allows fluorescent labeling to be limited to the nucleus of the cell in the synthesis period of the cell cycle, enabling identification. At 2, 4, 8 and 12 weeks after allograft, the level of fluorescence of cells with PKH-26 and BrdU was measured from 6 different microscopic views. Images on slide film were scanned into the computer, and the intensity of fluorescence was measured with NIH imaging software.

Histological evaluation: Consecutive frozen cross-sections about 6 µm thick were made from the intervertebral discs of recipients. After PKH-26 fluorescence was observed and photographed, the samples were stained with safranin O.

Results: Changes in disc space: There was progressive narrowing of the intervertebral disc space in the cell transplantation group, the carrier insertion group, and the non-insertion group. However, 2 weeks after the operation, there was a greater degree of narrowing in the non-insertion group and the carrier insertion group than in the cell transplantation group. At 8 weeks, the non-insertion group and the carrier insertion group showed narrowing of 28% and 44%, respectively, whereas the narrowing of the cell transplantation group was still less than 20% at 12 weeks; these are significant differences.

Viability and proliferation activity of allografted cells: In the cell transplantation group, fluorescence of PKH-26 was observed 2, 4, 8 and 12 weeks after allograft, indicating that the allografted cells had survived. Examination of cells for labeling of nuclei with BrdU showed similarly positive results 2, 4, 8 and 12 weeks after allograft. The ratio of BrdU-positive cells to PKH-26-positive cells was about 10%, and this ratio did not change significantly over time.

Histological findings: In the non-insertion group, there was scant cellularity in the NP, and only amorphous tissue weakly stained with safranin O was observed, and, in the AF, tissue with scant cellularity and little safranin O staining was observed. In the carrier insertion group, some cells had migrated into the carrier inside the NP, resulting in weak staining, and, in the AF, carrier surrounded by cicatrization tissue with scant cellularity and weak staining was observed. In the cell transplantation group, AF cells were observed adhering in the NP and the AF, and, inside and around the carrier, marked accumulation of cartilage like matrix with strong staining was observed.

Discussion: Nucleotomy or minimally invasive surgical procedures such as percutaneous nucleotomy or laser disc decompression inevitably exacerbate intervertebral disc degeneration, but attention is hardly ever paid to tissue repair at the time of surgery. Basic research on the prevention of intervertebral disc degeneration has been done by Nishimura et al.[4] Using a rat model in which herniations were induced in caudal vertebrae of rats and either fresh or frozen NP was injected, they report that the progression of intervertebral disc degeneration was suppressed. Okuma et al.[5] similarly report success in controlling progress of intervertebral disc degeneration in a rabbit experimental model with reinsertion of NP. Nishida et al.[6] report the possibility of treating degenerating intervertebral discs by introducing the TGF-β1 gene into NP cells of rabbits using an adenovirus as a vector. However, considering the fact that the pathology of intervertebral disc herniation includes degeneration of and damage to the AF, and that even minimally invasive surgical procedures cause additional damage to the AF and hasten degeneration, if the repair or regeneration of the AF cells surrounding and protecting the NP cells is incomplete, intervertebral disc degeneration caused by further protrusion of the NP or lack of NP cell adherence will lead inevitably to disc degeneration, even if it is possible to activate and regenerate NP cells after surgery.