Introduction
Recent advance of techniques in molecular biology has provided new knowledge on biologic nature of the intervertebral disc and disc cells. Experimental studies on disc cell function has enabled researchers and clinicians to develop new approaches for the treatment of disc degeneration and regeneration of the disc. Although recent studies addresses new approaches to repair degenerated discs, adequate method for broad clinical application has not yet been available. We have previously reported the regenerative effect of autologous mesenchymal stem cell transplantation in development of a technique to treat degenerative disc disease [1, 2]. Despite, the effectiveness of the procedure, its pathogenesis was unclear. The purpose of this study is to evaluate whether transplanted mesenchymal stem cells (MSCs) will survive and differentiate into cells expressing disc cell phenotypes after being transplanted to degeneration-induced discs in rabbits.

Materials and Methods
Forty-eight New Zealand white rabbits (ave. weight: 1.5Kg) equally into eight groups {normal control (n=6); degeneration-induced (n=6); and MSC transplantation models were evaluated at 2, 4, 8, 16, 24 and 48 weeks (n=36)}. Animal experiments were carried out under IRB approval. Induction of degeneration in degeneration-induced and MSC transplanted groups. Induction of disc degeneration in sham and MSC transplantation models, and collection of marrow was performed in MSC transplantation models at the same time. MSC isolation. Autologous MSCs were isolated from rabbit bone marrow by gradient isolation of mononuclear cells and cell attachment to tissue culture plastic as described before. The obtained MSCs were checked for possession of multi-lineage differentiation by adipogenic, chondrogenic and osteogenic differentiation assays. Infection of MSCs with lentiviral vector. Cultured MSCs progressing to transplantation were infected with lentiviral vector expressing GFP twice at MOI 20. Vector incorporation data was analyzed by quantitative analysis of the infected cells by flow-cytometry. Transplantation of MSCs. Gene labeled MSCs were embedded in Atelocollagen® gel solution at a final cell density of 5×10^5 cells/ml and transplantation was carried out immediately. 0.04 ml of mildly cooled MSC/Atelocollagen® implant in runny form was injected using an insulin micro-injector with a 27-gauge needle. Evaluation. At 2, 4, 8, 16, 24 and 48 weeks after transplantation, MRI and radiograph were performed in order to confirm disc regeneration and rabbits were euthanized. Frozen sections of the discs were made and double labeled with anti-GFP antibody and antichondroitin-4-sulphate, chondroitin-6-sulphate, keratan-sulphate, type I collagen and type II collagen. Double labeled sections were stained with specific second antibodies and examined under confocal laser scanning microscopy. Furthermore, aggrecan, versican, type I and II collagen mRNA expression in the disc at 48 weeks after transplantation were quantified by RT-PCR and compared among groups. Unpaired t-test was used for statistical analysis and considered P values <0.05 to be statistically significant.

Results
MRI and radiograph results showed significant restoration of water content level and disc height measurement in MSC transplanted discs compared to degeneration-induced group discs throughout all periods. Immunohistochemistry of the frozen section revealed that GFP-positive cell were detected in the nucleus pulposus throughout all periods with its percentage rising from 21 ± 6% in 2 week after transplantation to 55 ± 8% in 48 weeks after transplantation, which proved survival and proliferation of the transplanted MSCs (Fig.1). Confocal laser microscopic examination of the double-labeled sections showed positive staining of all proteoglycan epitopes and type II collagen in some of the GFP-positive cells. Cells showing positively for keratan-sulphate were most frequently observed (45 ± 6%)(Fig.2). Type I collagen stained cells were not observed in GFP positive cells. RT-PCR results demonstrated significant restoration of aggrecan, versican and type II collagen mRNA expression after MSC transplantation (Fig.3).

Discussion
There has been an increasing rise of interest in stem cell therapy, since it has provided new option in broad range of diseases. However, stem cell application to treat intervertebral discs has just begun [3-6]. Our previous studies hypothesized that regenerative effect of MSC transplantation is carried out by activation of original disc cells and/or by MSCs differentiating to cells presenting disc cell function. Here we provide evidence that transplanted MSCs not only survive but also proliferate and differentiate into cells expressing phenotypes of disc cells. Results of the current study demonstrate one possible explanation for regenerative process of MSC transplantation to the degeneration-induced disc.

References
5. Richardson S, et al. ISSLS, 2003