COMBINATION OF COCULTURE SYSTEM WITH MESENCHYMAL STEM CELLS AND AUTOLOGOUS PLATELET RICH PLASMA SIGNIFICANTLY ACTIVATES HUMAN NUCLEUS PULPOSUS CELLS

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Introduction
Lumbar disc degeneration is a common cause of chronic low back pain. Autologous disc cell transplantation, especially autologous nucleus pulposus (NP) cell transplantation is an attractive proposition in decelerating progression of disc degeneration. However, human NP cells are difficult to culture and expand. Therefore, establishment of a strategy to activate and expand NP cells in a short period is of great interest. Coculture with mesenchymal stem cells (MSCs) has proved its potential in accelerating the activation of human NP cells [1]. On the other hand, platelet rich plasma (PRP) has been reported to improve extracellular matrix metabolism in porcine NP cells with its rich growth factors [2]. In order to apply autologous NP cell transplantation clinically, most efficient and timesaving method should be considered. The purpose of this study is to investigate whether coculture with mesenchymal stem cells with or without combination of autologous PRP would maximize the effect of activating human NP cells in vitro.

Materials and Methods
Under informed consent, 3 patients were registered in the study. Human NP tissue was obtained under informed consent from surgical specimens. NP cells were isolated and released enzymatically by digestion with 0.05%trypsin for 1 hour and 0.025% collagenase for 2 hours at 37°C. MSCs were isolated from 50ml of marrow blood aspirated from the iliac crest and using density gradient separation of mononuclear cells. The adherent cells were regarded as autologous MSCs. The method of preparing the PRP was with a double-spin procedure, in the first phase, peripheral blood mixed with sodium citrate was centrifuged at 2000g 3 min, and in the second phase, the separated plasma fraction was recentrifuged at 5000g 5min. Platelets were concentrated and Platelet Poor Plasma (PPP) was removed, and PRP was added to 1/9 amount of autologous serum and medium. After 2 hours at normal temperature, the solution was coagulated and the clot of fibrin netting was crystallized into medium. This solution, the top of which was highly centrifuged and filtrated was regarded as 10% auto-PRP solution used for culture. A 12-well culture plates and inserts possessing polyethylene terephthalate membrane with 0.4µm pores at the bottom were used for culture. NP cells were seeded inside each culture insert at 1.0×10⁵cells. They were monocultured in DMEM with 10% auto-serum (group A) or monocultured in DMEM with 10% auto-PRP (group B) or cocultured having direct cell-to-cell contact with autologous MSCs in DMEM with 10% auto-serum (group C) or cocultured in DMEM with 10% auto-PRP (group D) (Fig.1). After 3, 5, and 7days of culture, they were evaluated for cell proliferation using WST-8 assay, DNA and proteoglycan (PG) synthesis by measuring the uptake of [³H]-thymidine and [¹⁵N]-sulphate. For measurement of DNA and PG content, cells were seeded in 1.2% alginate at 1×10⁶cells/ml and cultured for 3 and 5 days and evaluated by Hoechst 33258 and Dimethylmethylene Blue (DMMB) assay.

Result
In cell proliferation assay, after 7 days of culture, the average number of cells was 7.69×10⁵cells/well in group A, 22.48×10⁵cells/well in group B, and 11.36×10⁵cells/well in group C, and 22.56×10⁵cells/well in group D (Fig.2). In DNA and PG synthesis assay, the average DPM in 7days of culture measured for [³H]-thymidine was 0.23DPM/cell in group A, 2.22DPM/cell in group B, and 0.40DPM/cell in group C and 2.51DPM/cell in group D (Fig.3), the average DPM in 7days of culture measured for [¹⁵N]-sulphate was 0.10DPM/cell in group A, 1.05DPM/cell in group B, and 0.34DPM/cell in group C and 1.13DPM/cell in group D (Fig.4). The average of PG content per DNA calculated by DMMB and Hoechst 33258 after 5 days culture showed 2.79µg/µgDNA in group1, 5.60µg/µgDNA in group2, and 8.75µg/µgDNA in group3 (Fig.5).

Discussion
Platelets have several granules such as , , and -granules. When platelets response to the blood coagulation, these granules are released with cell stimulating growth factors such as PDGF, TGF-, EGF and VEGF. Result of the study showed that PRP upregulated the viability of NP cells more relevant than the coculture with MSCs. Additionally, the combination of coculture system with MSCs and autologous PRP was the most efficient, especially in early phase of culture. We suppose that using this technique, it may be clinically possible to obtain appropriate number of fresh NP cells in shorter term compared to the conventional techniques.

Conclusion
NP cells are significantly activated by combination of coculture system with MSCs and autologous PRP. Use of these two techniques shall be useful in conducting clinical application of reinsertion of activated NP cells.

Fig. 1 study design

Fig. 2

Fig. 3

Fig. 4

Fig. 5

References

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