INTRODUCTION
Platelet-rich plasma (PRP) is an autologous concentration of platelets in a small volume of plasma, and contains several kinds of autologous growth factors, such as transforming growth factor β1 (TGF-β1) and platelet-derived growth factor (PDGF) in high concentrations. Gelatin hydrogel microsphere is a biodegradable material which can immobilize these growth factors physicochemically and release them in a sustained manner in vivo. In the application of growth factors in vivo, their short biological half-life has been considered a serious drawback, but recently in the application of some growth factors such as osteogenic protein-1 into the intervertebral disc (IVD), the effectiveness of single injection has been reported (1). The objective of this study is to investigate the effect of combined injection of PRP and gelatin hydrogel microspheres into the degenerated IVD of rabbits, and compare the regenerative effects with the single injection of PRP without microspheres.

METHODS
This experimental procedure was approved by the Experimental Animal Center Committee at the author’s institution. Disc degeneration was induced in 36 male Japanese white rabbits weighing 2.8kg on the average, by the partial aspiration of the nucleus pulposus (NP) in L3-4, L4-5, and L5-6 discs. These rabbits were divided into 4 groups; namely, PRP-MS group (n=9), Phosphate-buffered saline (PBS)-MS group (n=9), PRP only group (n=9), and Sham group (n=9). Injection was performed 2 weeks after the aspiration of the NP. In the PRP-MS group and PRP only group, PRP was prepared by the centrifugation of fresh blood at 2 weeks after the aspiration of the NP. In the PRP-MS group, PRP-impregnated gelatin microspheres were injected into the NP of degenerated discs. In the PBS-MS group, PBS-impregnated gelatin microspheres were injected in the same manner. In the PRP only group, the same amount of PRP without microspheres was injected. In the Sham group, only needle puncture was performed. At 2, 4, and 8 weeks after the injection, IVDs of each rabbit were removed and evaluated histologically by H-E staining, and immunohistochemically by immunostaining for proteoglycan. A previously described histological grading scale for the NP and annulus fibrosus (AF) was also used (Grade 0 (Normal), 1, 2, 3, 4, 5: NP: Complete replacement, AF: Indistinct). Electron microscopy was also employed to examine the morphological changes in the NP cells after injection of PRP. All data were analyzed by the Mann-Whitney U test and the Kruskal-Wallis test, and statistical significance was considered when the p value was less than 0.05.

RESULTS
In the PRP-MS and PRP only group, the purified PRP contained about 35 times the number of platelets in the whole blood. Histological examination revealed that numerous NP cells and extracellular matrix existed throughout the experiment in the PRP-MS group (Fig.1, right). Otherwise in the PBS-MS (Fig.1, left) and PRP only (Fig.1, middle) groups, NP cells completely disappeared and proliferating connective tissue invaded into the NP at 8 weeks after injection. At 8 weeks after the injection, almost every microsphere was fragmented or disappeared. Neither ossification nor invasion by inflammatory cells was observed in any group. Regarding the histological grading scale, the grade of degeneration in the NP and the AF in the PRP-MS group at 8 weeks after injection was significantly less severe than that of the other groups (Fig.2). Immunohistochemical staining for proteoglycan revealed that intense and regular positive immunostaining for proteoglycan was observed in the NP of the AF in the PRP-MS group (Fig.3, right). Otherwise only weak immunostaining was observed in the PBS-MS (Fig.3, left) and PRP only (Fig.3, middle) groups. Transmission electron microscopy demonstrated that there was no ultrastructural difference between the normal control NP cells and the NP cells at 8 weeks after injection of PRP-impregnated microspheres.

DISCUSSION
In this experiment, we prepared gelatin hydrogel microspheres that can immobilize TGF-β1 and PDGF ionically, and release them in a sustained manner in vivo as the microsphere degrades (2)(3). It was reported that this gelatin hydrogel had been a substance capable of activating platelet growth factors such as TGF-β1 and PDGF (4). Our histological results suggest that the PRP growth factors immobilized in microspheres were released continuously in the NP, and suppressed the progression of IVD degeneration. Moreover, our immunohistochemical results suggest that the proteoglycan synthesis was up-regulated by the combined injection of PRP and gelatin hydrogel microspheres. It is likely that the activation of PRP growth factors was not induced or maintained by the single injection of PRP. In this experiment, no obvious side effects such as ossification, inflammation, and mutagenic change were observed. We concluded that the gelatin hydrogel microsphere may be useful for the IVD regeneration using PRP, and the combined injection of PRP and gelatin hydrogel microspheres to the NP can be an effective and safe therapeutic modality for IVD degeneration.

REFERENCES

THE EFFECT OF BIODEGRADABLE GELATIN HYDROGEL MICROSPHERES ON THE INTERVERTEBRAL DISC REGENERATION USING PLATELET-RICH PLASMA

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