

**Ultra-purified alginate gel implantation decreases inflammatory cytokine levels, prevents intervertebral disc degeneration, and reduces acute pain after discectomy**

Katsuro Ura<sup>1</sup>, Katsuhisa Yamada<sup>1\*</sup>, Takeru Tsujimoto<sup>1</sup>, Daisuke Ukeba<sup>1</sup>, Norimasa Iwasaki<sup>1</sup>, Hideki Sudo<sup>2\*</sup>

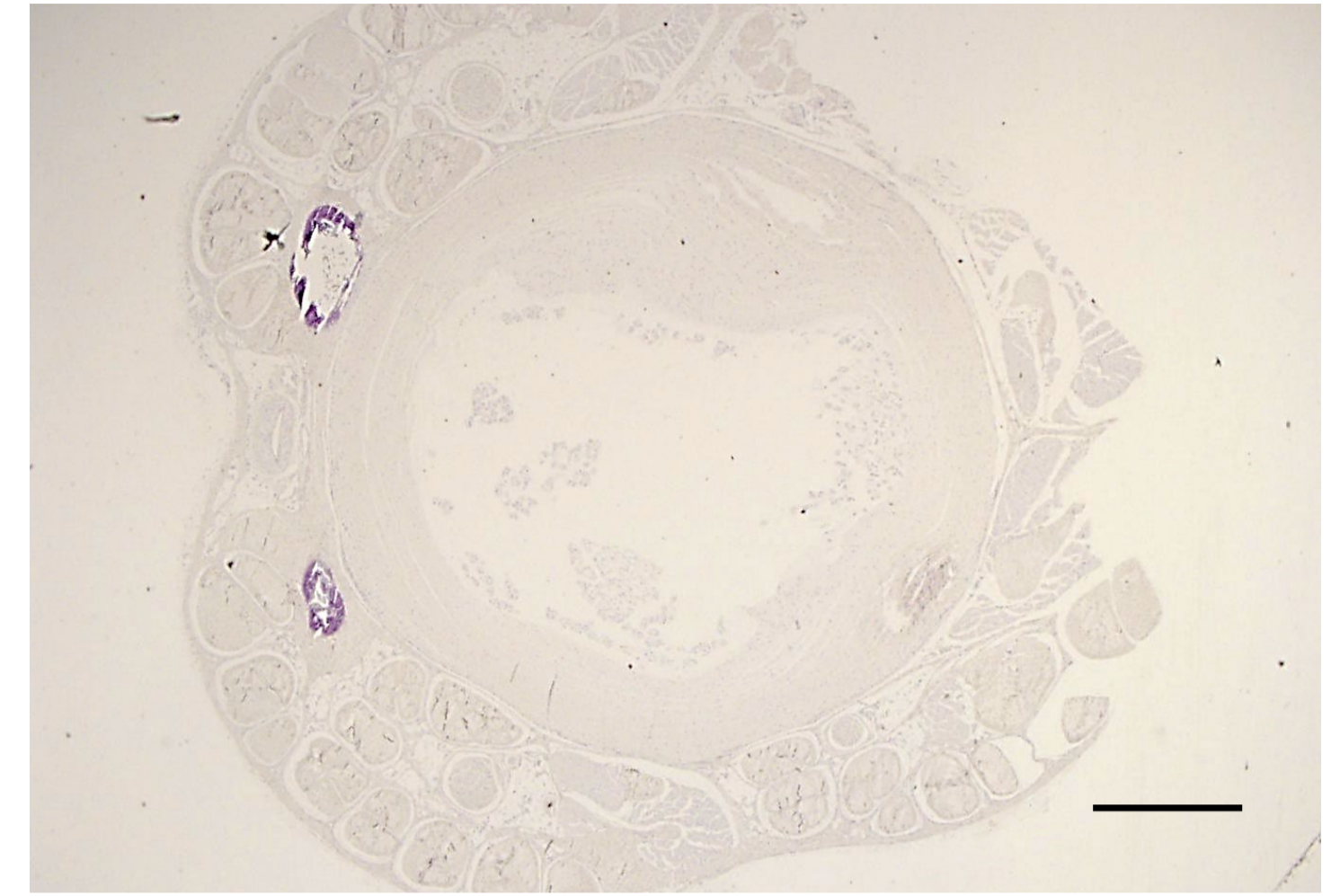
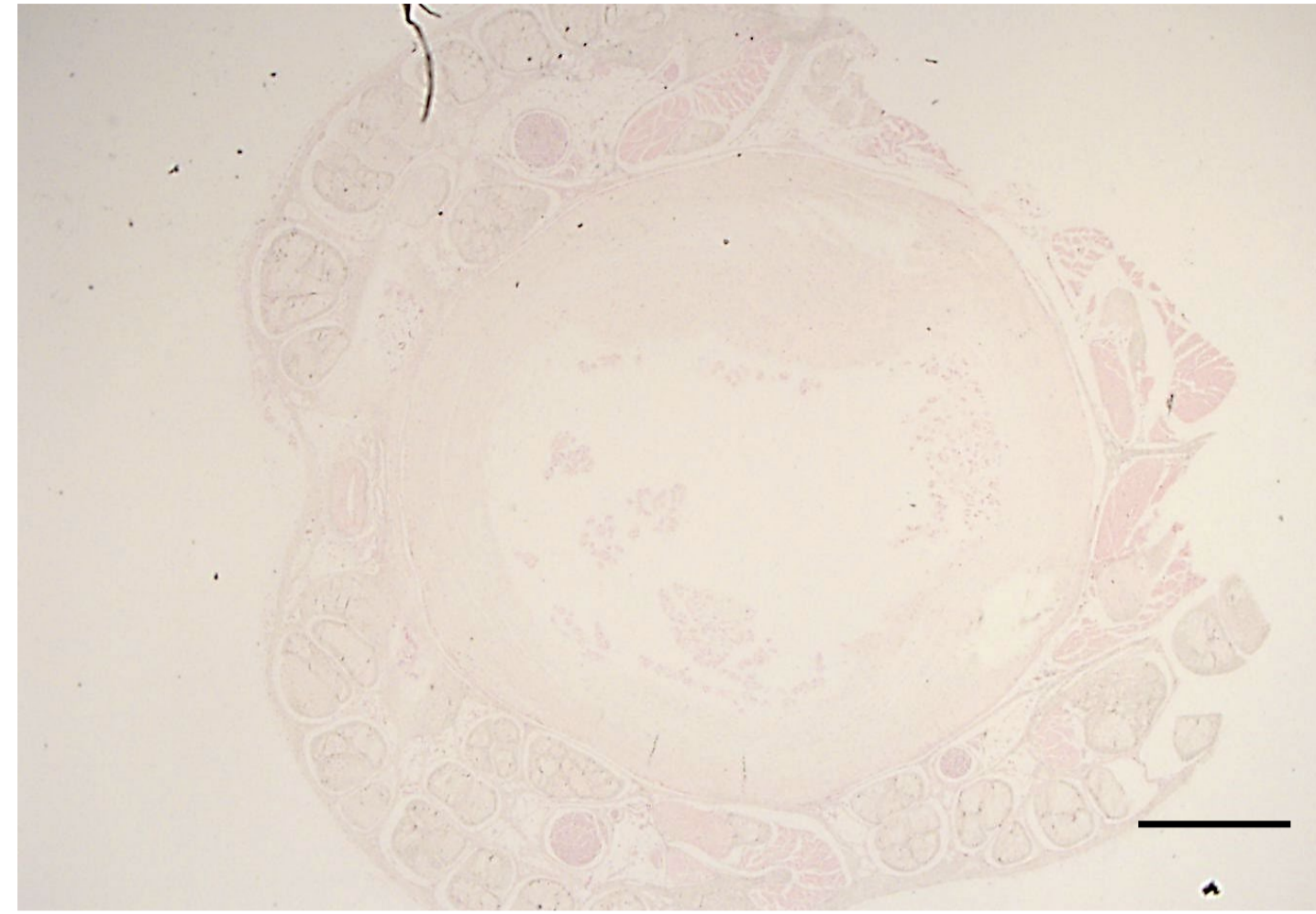
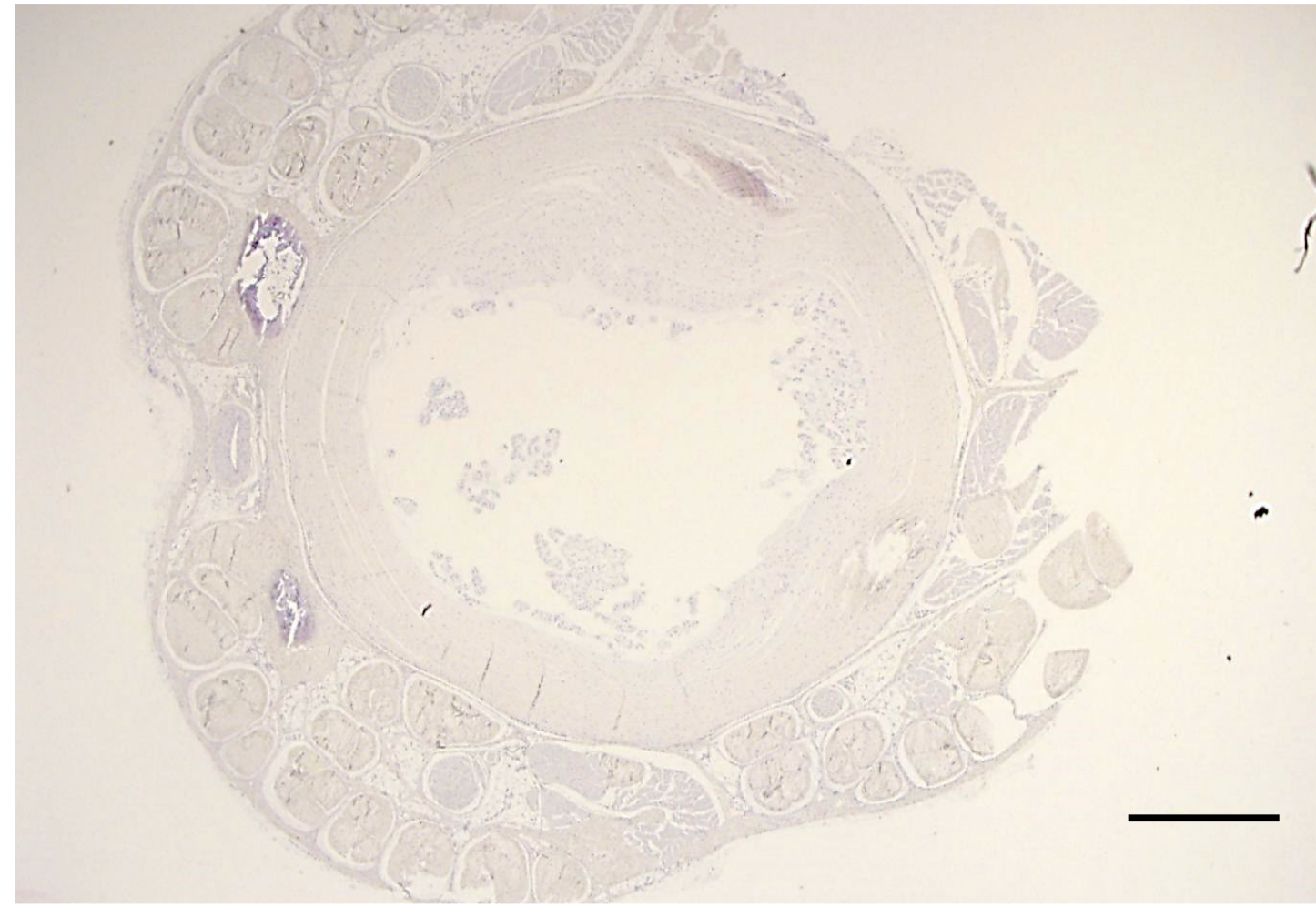
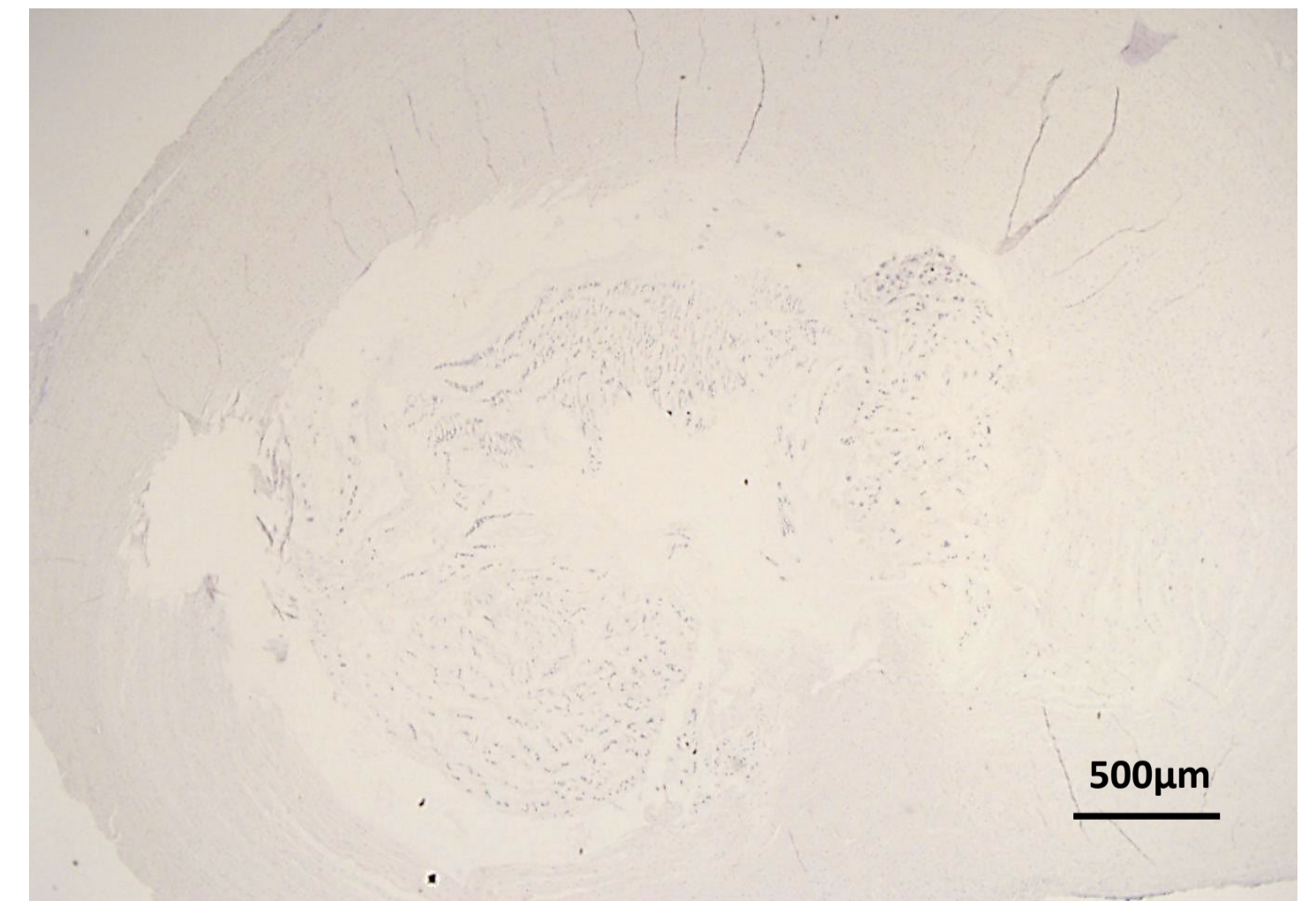
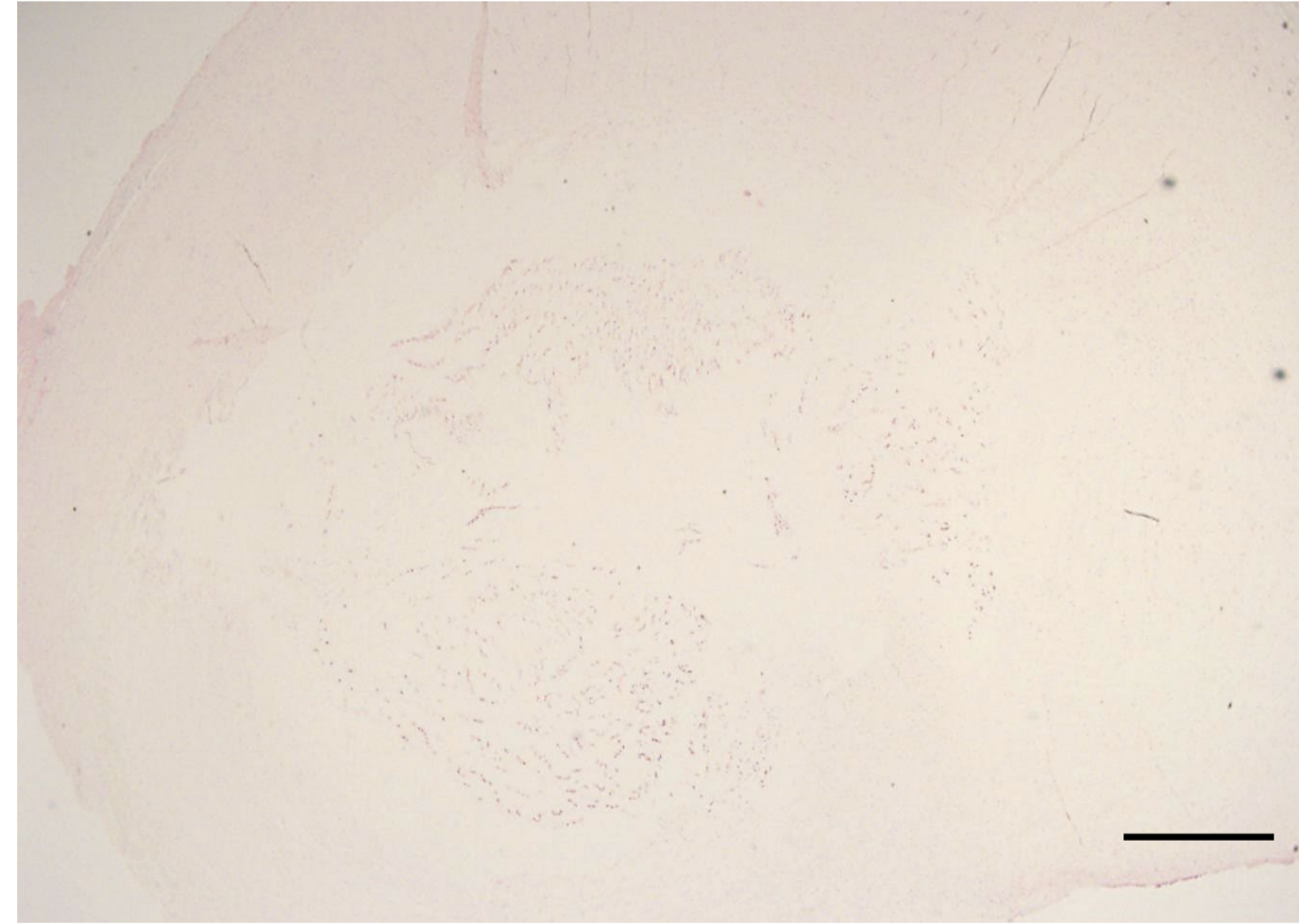
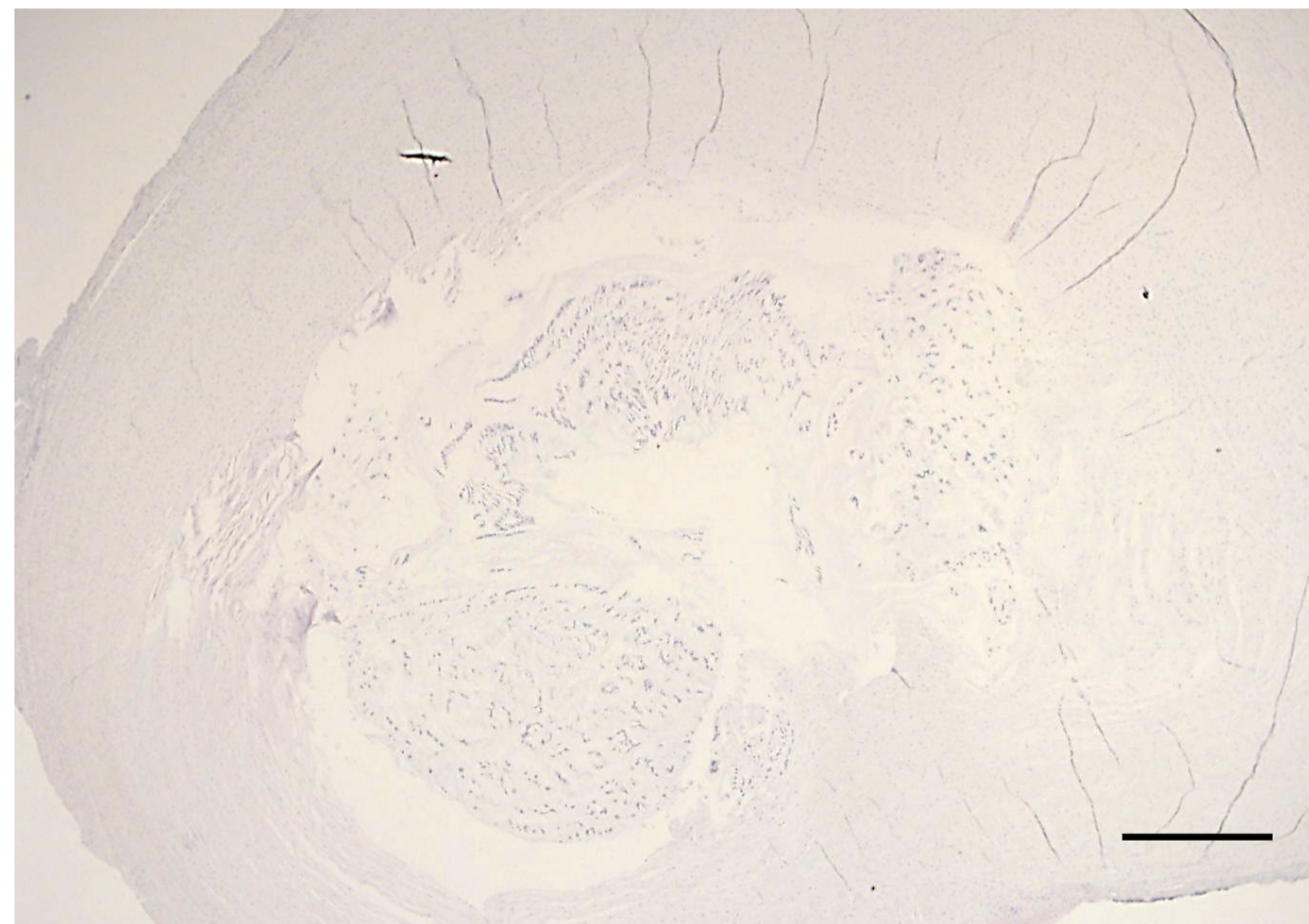
<sup>1</sup> Department of Orthopedic Surgery, Faculty of Medicine and Graduate of Medicine, Hokkaido University, Sapporo, Hokkaido, Japan

<sup>2</sup> Department of Advanced Medicine for Spine and Spinal Cord Disorders, Faculty of Medicine and Graduate of Medicine, Hokkaido University, Sapporo, Hokkaido, Japan

**\*Corresponding Author:**

Katsuhisa Yamada, MD, PhD, Department of Orthopedic Surgery, Faculty of Medicine and Graduate of Medicine, Hokkaido University, N15W7, Sapporo, Hokkaido 060-8638, Japan; Tel: 81-11-706-5934, Fax: 81-11-706-6054, E-mail: yka2q@yahoo.co.jp

Hideki Sudo, MD, PhD, Department of Advanced Medicine for Spine and Spinal Cord Disorders, Faculty of Medicine and Graduate of Medicine, Hokkaido University, N15W7, Sapporo, Hokkaido 060-8638, Japan; Tel: 81-11-706-5934, Fax: 81-11-706-6054, E-mail: hidekisudo@yahoo.co.jp

**TNF- $\alpha$** **IL-6****TrkA****Rat****Rabbit**

**Supplementary fig. 1** Negative controls for TNF- $\alpha$ , IL-6 and TrkA in immunohistochemistry. TNF- $\alpha$  -positive cells are stained red and nuclei was stained purple using hematoxylin. IL-6 -positive cells are stained green and nuclei was stained red using nuclear fast red. TrkA -positive cells are stained brown and nuclei was stained purple using hematoxylin. Scale bar, 500  $\mu$ m.

**Supplementary Table 1. Histological grading**

Category	Score		
	Sham	Punch	Gel
Endplate	0	0.67 ± 0.47	0.17 ± 0.37
AF	0	1	0.33 ± 0.47
Boundary between NP and AF	0	1.67 ± 0.47	0.17 ± 0.37
Cellularity of NP	0	2	0.5 ± 0.5
Matrix NP(H&E)	0	2	0.5 ± 0.5
Matrix NP(SO)	0	2	0
Total score	0	9.33 ± 0.94	1.67 ± 1.89

N = 6 discs in each group, mean ± standard deviation. NP; nucleus pulposus. AF; annulus fibrosus. H&E; hematoxylin and eosin. SO; safranin O