



Supplementary Material

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Methods

Histology

Three coronal sections from each sample were stained with toluidine blue. The thickness of the calcified layers and articular cartilage of the medial tibial plateau was measured further on these sections. The scoring and measurement processes were also performed in blinded fashion and the mean of the three sections served as the datum for that sample.¹

Immunohistochemical assessments

In cartilage tissue of the tibial plateau, the mean intensity of optical density (IOD), given in IOD/mm², was defined here as the sum of the integrated optical density divided by the area of the cartilage tissue in the region of interest (ROI) under a magnification of 10×. All sections were measured by Image Pro Plus (IPP) version 6.0 software (Media Cybernetics, Rockville, Maryland, USA).^{1,2}

TUNEL staining

Articular cartilage was assessed by TUNEL assay using an in situ apoptosis detection kit (Merck Millipore, Burlington, Massachusetts, USA), according to the manufacturer's instructions. Chondrocyte apoptosis was determined as the percentage of TUNEL-positive chondrocytes in the middle region of the medial tibia, by using the IPP software.^{3,4}

References

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2. **Gou Y, Tian F, Kong Q, et al.** Salmon Calcitonin Attenuates Degenerative Changes in Cartilage and Subchondral Bone in Lumbar Facet Joint in an Experimental Rat Model. *Med Sci Monit*. 2018;24:2849-2857.
3. **Iijima H, Aoyama T, Ito A, et al.** Effects of short-term gentle treadmill walking on subchondral bone in a rat model of instability-induced osteoarthritis. *Osteoarthritis Cartilage*. 2015;23(9):1563-1574.
4. **Bei M, Tian F, Liu N, et al.** A Novel Rat Model of Patellofemoral Osteoarthritis Due to Patella Baja, or Low-Lying Patella. *Med Sci Monit*. 2019;25:2702-2717.