

Partial patellectomy induces a decrease in the proteoglycan content in the remaining patellar articular cartilage. An experimental study in rabbits

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ABSTRACT

We studied the alterations and distribution of the proteoglycan (PG) content of the remaining patellar articular cartilage after unilateral partial patellectomy in 13 rabbits. Sagittal sections of the patella were prepared and stained with Safranin O for quantification of changes in the PG content of the patellar articular cartilage using a commercially available imaging analysis system. Our findings suggest that partial patellectomy results in a decreased PG content in the remaining patellar articular cartilage. In addition, the postoperative development of metaplasia in the scar tissue next to the healing interface may represent a compensatory response, which could prevent a further reduction in the PG content and hence the development of osteoarthritis in the remaining patellar articular cartilage.

Introduction

Partial patellectomy is clinically indicated for the treatment of severe comminuted fractures (1). However, partial patellectomy shortens the extensor mechanism and decreases the patello-femoral contact areas, subsequently resulting in an increased contact pressure of the patello-femoral joint (2). Previous studies have demonstrated that unphysiological joint loading such as that due to anterior cruciate ligament transection (3), joint disuse (4), and impact or excessive joint loading (5), results in a decreased PG content in the cartilage matrix, which has been regarded as a causative factor in the development of chondromalacia and osteoarthritis (OA). The present study was designed to investigate the effect of partial patellectomy on the PG content and the potential development of OA in the articular cartilage of the remaining patella in rabbits.

Material and methods

Animals and partial patellectomy

Under general anesthesia partial patellectomy was performed by removing the distal third of the patella using a unilat-

eral transverse osteotomy in 13 mature female New Zealand white rabbits (1). The operated knee was immobilized for 4 weeks and the contralateral non-operated knee served as the control. Animals were sacrificed at week 8 (n = 5), week 12 (n = 4), and week 24 (n = 4) post-operatively for histology and imaging quantification.

The experimental procedures were drawn up in compliance with the guidelines contained in the "Care and Use of Animals" published in the Information for Authors of *American Journal of Physiology*. This study was approved by the Animal Research Ethics Committee of the Chinese University of Hong Kong.

Histomorphology

After decalcification and embedding in paraffin, 5 sections (each 5 µm thick) from the mid-medial sagittal plane of each patella were cut. One of the sections was stained with H&E for general histological study (6) and the remaining 4 sections were stained with Safranin O for image quantification of their PG content (7).

Image quantification

The histological sections were digitized and assessed using a Leica Q5000MC imaging analyzer (Leica Cambridge Ltd., Cambridge, UK). The Safranin O intensity expressed as pixel density ranging from 0 to 255 (from dark to bright) was measured in the superficial and deep zones of the remaining articular cartilage matrix and in the corresponding regions of the contralateral controls. Since the pixel density is a linear function, which is dissimilar to the logarithmic function characterizing the true optical density or absorbance, these values were converted mathematically to a quantitative optical density (OD) value using the formula $OD = \log_{10} (255 / [255 - X])$, where X represents the mean pixel density (8). In addition, a reliability test for measuring the batch to batch variations was performed in order to obtain the coefficient of variation (CV) in the Safranin O staining intensity among 4 mid-medial sagittal sections of individual control specimens.

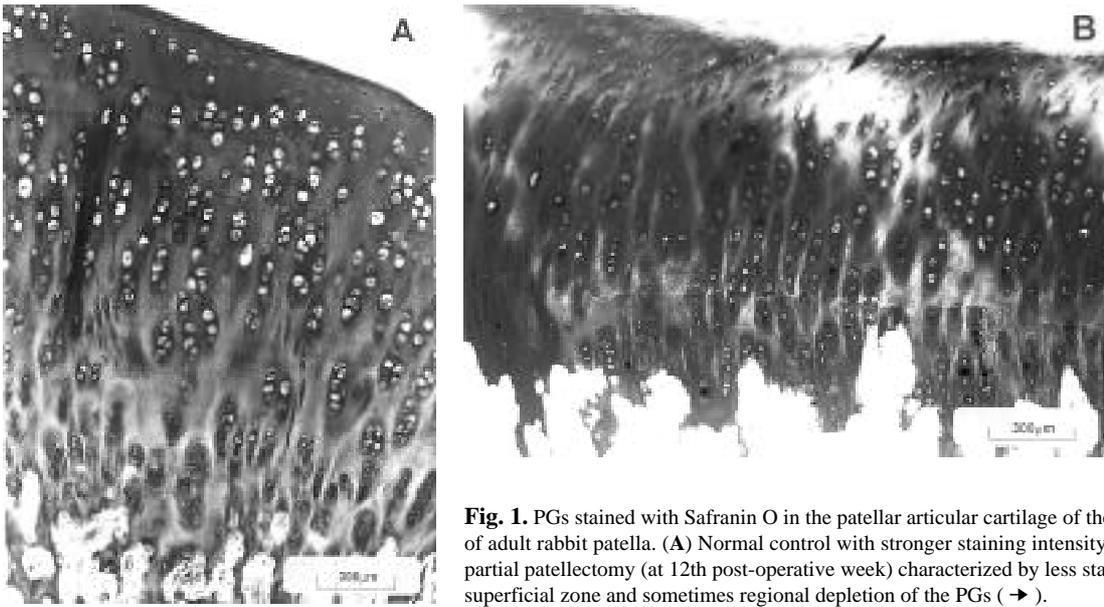


Fig. 1. PGs stained with Safranin O in the patellar articular cartilage of the medial mid-sagittal section of adult rabbit patella. (A) Normal control with stronger staining intensity; (B) remaining patella after partial patellectomy (at 12th post-operative week) characterized by less staining intensity mainly in the superficial zone and sometimes regional depletion of the PGs (→).

Statistical analysis

The T-test was used to compare the Safranin O staining intensity of the remaining patellar articular cartilage in the experimental groups with that of the contralateral controls. ANOVA was used to evaluate the percentage changes in Safranin O staining intensity measured at three healing time points as well as in the superficial and deep zones between the experimental and control specimens. The significance level was set at $p = 0.05$.

Results

Histomorphology

In control specimens, the PGs were distributed throughout the cartilage zone, being more densely concentrated around the lacuna of the chondrocytes (Fig. 1A). However, the staining intensity was decreased, mostly with regional disaffinity of Safranin O staining in the superficial zone of the remaining articular cartilage after partial patellectomy (Fig. 1B). There were signs of degenerative and regenerative changes in the cartilage matrix of the remaining patella next to the healing interface, mainly observable in experimental specimens harvested at week 8 and week 12. Metaplasia developed, with healing over time, in the scar tissue next to the healing interface (Fig. 2).

Imaging quantification of the PG content

The CV of the reliability test was 7.6%.

T-test analysis showed that the PG content decreased significantly after partial patellectomy ($p < 0.001$). ANOVA analysis revealed a significant post-operative reduction in the PG content of the remaining articular cartilage, which was greater in the superficial zone (mean 30.9%) than in the deep zone (mean 19.4%) ($p < 0.01$) at three healing time points. The PG content tended to return to its original values, however, without statistical significance in both the superficial and deep zones (Fig. 3).

Discussion

Compared with chemical methods (3, 9), imaging quantification provides a reliable approach to assess the spatial distribution of PG and its relationship to cartilage degradation (3, 10). The imaging quantification procedure used in our study is reliable as confirmed by the CV of 7.3% in Safranin O staining intensity, which is within the reported range, and any changes exceeding $2.8 \times$ CV of the applied method can be considered as true or significant changes in the study variables (9, 10).

The results of our study show that the PG content of the remaining patellar articular cartilage decreases significantly after partial patellectomy. The decreased PG content may be associated with two major factors. Firstly, it may be related to the degeneration of chondrocytes next to the healing interface in the early phase of healing causing the reduced produc-

tion of PGs in the affected region. Secondly, it may be due to a decreased patello-femoral contact area and a simultaneously increased contact pressure (2). Increased or decreased joint contact stress inducing a decrease in the PG content has been reported in studies using other experimental models (3-5).

The PG content tended to recover post-operatively in our experiment, although without a statistically significant difference among three healing time points. According to a power analysis ($\alpha = 0.20$ or a power of 0.80), even if the sample size were increased four times, no statistical significance would be achieved due to large individual variations in the PG content. This may be associated with variations in the precision of the excising distal third of the patella, surgical trauma, post-operative activities, local nutritional status, and (not the least important) the development of cartilaginous metaplasia in the scar tissue next to the healing interface of the articular surface in the remaining patella. Tendon cells are known to be capable of undergoing metaplasia and producing a cartilage-like structure along the length of tendons at regions subjected to compressive forces (11). As patello-femoral symptoms are generally stress-related, the development of metaplasia may partially contribute to an increased patello-femoral contact area and so to decreased contact pressure in the remaining patella with healing over time.

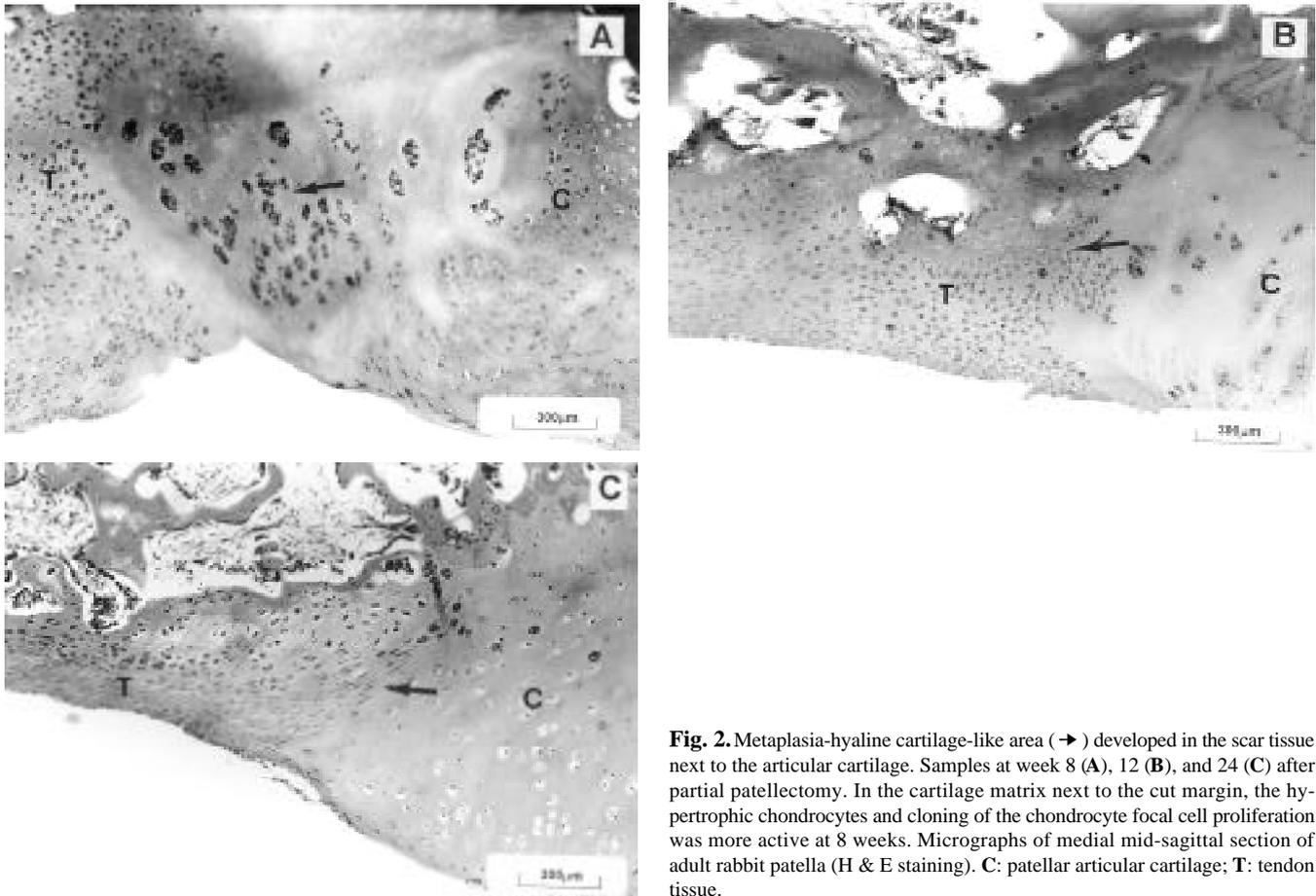


Fig. 2. Metaplasia-hyaline cartilage-like area (→) developed in the scar tissue next to the articular cartilage. Samples at week 8 (A), 12 (B), and 24 (C) after partial patellectomy. In the cartilage matrix next to the cut margin, the hypertrophic chondrocytes and cloning of the chondrocyte focal cell proliferation was more active at 8 weeks. Micrographs of medial mid-sagittal section of adult rabbit patella (H & E staining). C: patellar articular cartilage; T: tendon tissue.

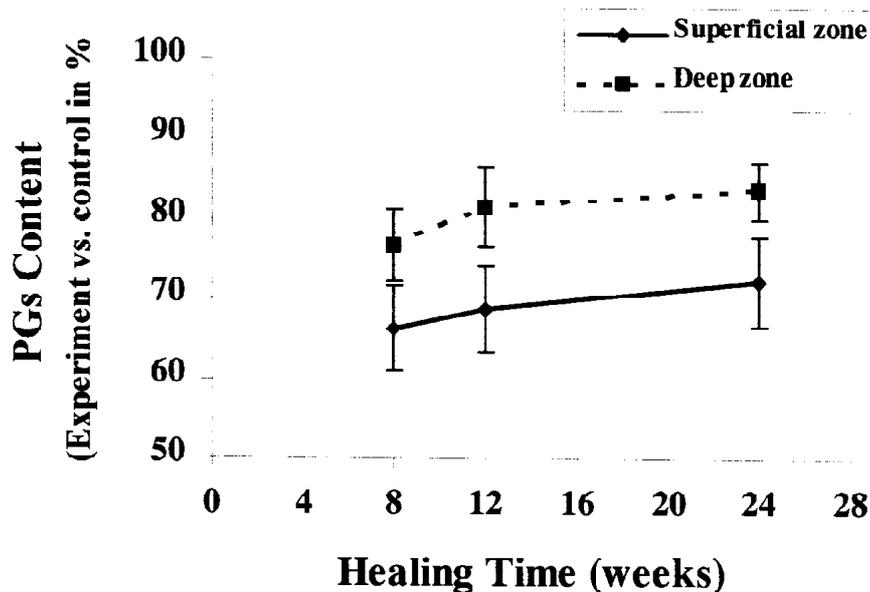


Fig. 3. Percentage decrease in the proteoglycan (PG) content of the remaining patellar articular cartilage after partial patellectomy, compared with the contralateral control. The decreased PG content in the superficial zone was significantly greater than that in the deep zone ($p < 0.001$). The PG content tends to return to normal levels, however without a statistically significant difference among the three healing time points in the superficial ($p = 0.29$) and deep zones ($p = 0.11$).

The gradually increased patello-femoral contact area of the remaining patella caused by the development of cartilaginous metaplasia may help to prevent the development of OA, as in addition no obvious surface fibrillation or frisure was observed in the remaining patella after partial patellectomy. However, excessive and uneven joint contact pressures have been regarded as causative factors in the development of patello-femoral or femoro-tibial chondromalacia and osteoarthritis after partial patellectomy (1, 12). The difference in the degrees of articular cartilage damage or OA development between our experimental study and clinical observations may be explained by the fact that pre-existing trauma frequently occurs to the articular cartilage at the moment of high impact injury in patients (1,12), while no such impact damage was generated in our partial patellectomy experiment, thus creating no trauma to the knee joint of rabbits. Therefore, partial patellectomy as used in the present study only resulted in early signs of chondromalacia or subclinical OA.

References

1. HUNG LK, LEE SY, LEUNG KS, CHAN KM, NICHOLL LA: Partial patellectomy for patellar fracture: Tension band wiring and early mobilization. *J Orthop Trauma* 1993; 7: 252-60.
2. MARDER RA, SWANSON TV, SHARKEY NA, DUWELLUS PJ: Effects of partial patellectomy and reattachment of the patellar tendon on patellofemoral contact areas and pressures. *J Bone Joint Surg* 1993; 75A: 35-45.
3. GUILAK F, RATCLIFFE A, LANE N, ROSENWASSER MP, MOW VC: Mechanical and biochemical changes in the superficial zone of articular cartilage in canine experimental osteoarthritis. *J Orthop Res* 1994; 12: 474-84.
4. SAAMANEN AM, TAMMI M, JURVELIN J, KIVIRANTA I, HELMINEN HJ: Proteoglycan alterations following immobilization and remobilization in the articular cartilage of young canine knee (stifle) joint. *J Orthop Res* 1990; 8: 863-73.
5. DONOHUE JM, BUSS D, OEGEMAR RT: The effects of indirect blunt trauma on adult canine articular cartilage. *J Bone Joint Surg* 1983; 65A: 948-57.
6. BANCROFT JD, STEVENS A: *Theory and Practice of Histological Techniques*, Vol. 1, 4th ed., Edinburgh, Churchill Livingstone, 1982; 309-39.
7. KIVIRANTA I, JURVELIN J, TAMMI M, SAAMANEN AM, HELMINEN HJ: Microspectrophotometric quantification of glycosaminoglycans in articular cartilage sections stained with safranin O. *Histochem* 1985; 82: 248-55.
8. O'KEEFE RJ, CRABB ID, PUZAS JE, ROSIER RN: Effects of transforming growth factor beta and fibroblast growth factor on DNA synthesis in growth plate chondrocytes are enhanced by insulin-like growth factor I. *J Orthop Res* 1994; 12: 299-310.
9. KIRALY K, LAPVETELAINEN T, AROKOSKI J: Application of selected cationic dyes for the semiquantitative estimation of glycosaminoglycans in histological sections of articular cartilage by microspectrophotometry. *Histochem J* 1996; 28: 577-90.
10. MARSHALL KW, CHAN AD: Arthroscopic anterior cruciate ligament transection induces canine osteoarthritis. *J Rheumatol* 1996; 23: 338-43.
11. OKUNDA Y, GORSKI JP, AN KN, AMADIO PC: Biochemical, histological and biomechanical analysis of canine tendon. *J Orthop Res* 1987; 5: 60-8.
12. FELLER JA, BARTLETT RJ: Patellectomy and osteoarthritis: Arthroscopic findings following previous patellectomy. *Knee Surg Sports Traumatol Arthrosc* 1993; 1: 159-61.