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Presence and mechanism of knee articular cartilage degeneration after meniscal reconstruction in dogs

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Summary

Objective: Partial meniscectomy is the golden standard for treating a bucket-handle tear in the meniscus of the knee, but it inevitably leads to articular cartilage degeneration. Surgical creation of an access channel between the lesion and the vascularized synovial lining is intended to induce ingrowth of repair tissue and thus avoid degeneration of articular cartilage.

Design: The presence and mechanism of cartilage degeneration were evaluated in 24 canine menisci after a longitudinal lesion and access channel had been created in the avascular part of the meniscus. In 12 menisci the channel was implanted with a porous polymer scaffold, while the remaining 12 were left empty. Evaluation was performed using routine histology and antibodies directed against denatured type II collagen (Col2-3/4M).

Results: Articular degeneration was apparent in the polymer implant group and the empty channel group. This consisted of fibrillation, loss of chondrocytes and decreased proteoglycan content. Areas of fibrillated cartilage always showed positive labeling with the collagen degradation antibody Col2-3/4M. Collagen degradation was also visible in non-fibrillated areas. The upper zone of the cartilage showed swelling especially in the implant group, with empty cell lacunae and moderate levels of Col2-3/4M antibody labeling.

Discussion: This reconstruction technique cannot be considered superior to partial meniscectomy. We propose that degradation of the collagen type II network is a result of cartilage fibrillation and vice versa. © 2003 OsteoArthritis Research Society International. Published by Elsevier Science Ltd. All rights reserved.

Key words: Dog, Meniscus reconstruction, Cartilage degeneration, Collagen degradation.

Introduction

The knee menisci are wedge-shaped semilunar discs of fibrocartilage interposed between the condyles of the femur and tibial plateau. Tears in the meniscus are often located in the inner avascular zone of the meniscus and are therefore not capable of spontaneous healing[1]. In these cases, treatment with partial meniscectomy is the golden standard. However, partial meniscectomy leads to degenerative changes in the articular cartilage; the degree of change appears to be directly related to the amount of meniscus removed[2]. If this tear could be induced to heal, partial meniscectomy would not be necessary and more importantly, articular cartilage degeneration might be avoided. Several different techniques have been devised to induce healing of a meniscal tear, e.g. the creation of a small access channel in the meniscal tissue, which connects the vascularized synovial lining of the knee joint with the meniscal lesion. This could potentially stimulate the ingrowth of fibrovascular repair tissue into the lesion[1].

Previously, the authors reported that creating a larger access channel and implanting a biodegradable porous polymer scaffold into this channel further improves the healing process[3,4]. Until now, the effect of this reconstruction technique on the prevention of articular cartilage degeneration has not been studied in detail.

The mechanism of articular cartilage degeneration is not completely understood. Collagen type II degradation seems to play an important role in this degeneration process[5,6]. Disruption or disorganization of the highly structured fibril network in the superficial cartilage might be an initiating factor in the degeneration cascade and might eventually lead to weakening and fibrillation of the superficial cartilage layers. In mice and rats, Stoop *et al.* showed that degradation of collagen type II is involved in the first stages of osteoarthritis (OA), using immunolocalization[7,8]. Price *et al.* demonstrated the same feature in humans[9]. This raises the question as to whether collagen type II degradation also occurs after meniscal reconstruction and whether it is related to the presence of morphological cartilage damage.

In this study, a bucket-handle lesion was created in the avascular zone of 24 canine menisci. An access channel was opened between the lesion and the vascularized

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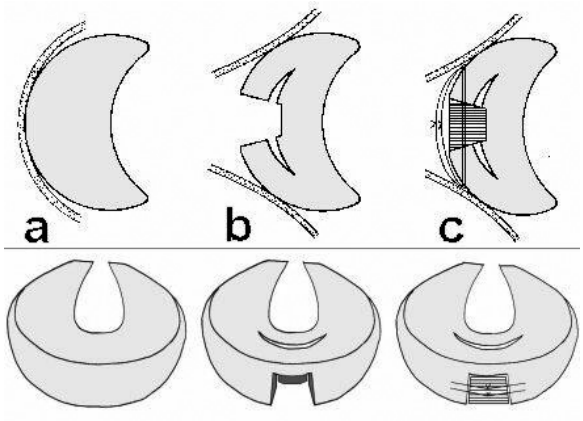

Fig. 1. Diagram of a normal lateral meniscus in the dog: upper view and three dimensional presentation (a). The dotted structure represents the joint capsule (outer line) and the synovial layer (inner line) of the knee joint. This was incised and opened (b). A full thickness longitudinal lesion was created in the avascular part of the meniscus (b). A partial thickness channel was created connecting the vascular periphery to the lesion in the avascular centre of the meniscus. A polymer implant was sutured into the channel using two sutures; the longitudinal lesion was not sutured (c).

synovial lining to induce healing. In 12 cases a porous polymer scaffold was implanted in this channel with the aim of enhancing the healing.

The purpose of this study was to determine in detail the consequences of this reconstruction technique on the articular cartilage degeneration in dogs. The presence of cartilage degeneration and the role of collagen type II degradation was evaluated by means of routine histology and antibodies directed against denatured type II collagen (COL2-3/4M)[10].

Material and methods

Experiments were performed under aseptic conditions on 24 lateral menisci in 12 adult male and female Beagles with an average weight of 13.1 kg (S.D.: ±1.6 kg). The institutional animal welfare committee approved all procedures.

In preparation for surgery, anesthesia was accomplished by intravenous administration of pentobarbital (30 mg/kg) and maintained after intubation with nitrous oxide (1:1) and isoflurane (0.5%). The right and/or left knee joint was entered using a lateral skin incision. Great care was taken to prevent damage to the collateral and cruciate ligaments and the articular cartilage.

To imitate a meniscal bucket-handle tear, a full thickness longitudinal lesion was created in the avascular part of the lateral meniscus (Fig. 1). In all cases, a partial thickness defect was created, occupying 30% of the meniscal length of the meniscal mid-substance to form an access channel between the vascular synovial lining and the lesion in the avascular zone of the meniscus.

In 12 dogs, a polymer implant was sutured into the partial thickness defect in the meniscus (access channel with implant, ACI), while on the contralateral side the meniscal defects were left empty (empty access channel, ACE). Both sides of the polymer implant were sutured to the meniscus with two 3-0 Ethilon sutures along the peripheral side of the implant. The longitudinal lesion was not sutured. The capsule and skin were closed in layers using 3-0 vicryl sutures. The dogs were allowed to walk as soon as possible. Cartilage degeneration was evaluated after three and six months.

POLYMER

Implants consisted of biodegradable polyester urethanes based on L-lactide/ε-caprolactone as described previously, which only yield non-toxic degradation products[11]. The properties of the polymers were improved compared with the polymers used in the earlier study[3]. The new polymers express many reactive carboxylic groups at the surface during degradation, which may increase the attachment between the polymer and the meniscal tissue. The polymer contained macropores with the size of 155–355 μm. To increase the ingrowth of repair tissue, these pores were interconnected with micropores of at least 30 μm, as suggested in earlier studies[12,13]. The porosity was 80% and the compression modulus was 200 kPa.

HISTOLOGY

Two sets of 12 knee joints were dissected after killing an equal number of dogs at 3 and 6 months post-surgery. Directly opposite the defect region in the meniscus the lateral tibia plateau was divided into an anterior half and a posterior half. The anterior part was processed for routine histology and the posterior part for immunohistochemistry.

The femur condyles and anterior halves of the lateral tibial plateau cartilage were fixed in 4% formaldehyde buffered with 0.1 M phosphate buffer (pH 7.4) and decalcified in 10% EDTA (Titriplex III, Merck, Darmstadt, Germany). After extensive rinsing with tap water, tissue blocks were dehydrated in alcohol and embedded in polymethylmethacrylate for sectioning. Sections (7 μm) were made in the plane through the cartilage opposite the defect and stained with haematoxiline-eosine (HE) and Toluidine blue.

IMMUNOHISTOCHEMISTRY

The posterior halves of the tibial plateau cartilage were decalcified without previous chemical fixation in 10% EDTA (Titriplex III, Merck, Darmstadt, Germany) and 7.5% polyvinylpyrolidone (PVP, Mr 29 000, Serva, Brinswich, Amsterdam, the Netherlands) in 0.1 M Tris buffer for 8 weeks at 4°C[14,15]. After extensive rinsing with 7.5% PVP in 0.1 M Tris buffer, tissue blocks were rapidly frozen in liquid nitrogen and stored at −70°C. Coronal sections were cut (7 μm) on a Bright 3050 cryostat and mounted on glass microscope slides pre-coated with 3-aminopropyltriethoxysilan (Sigma, St Louis, MO). Sections were made in the plane through the cartilage opposite the defect and were dried for 1 h and stored at −80°C until required for further use. After thawing, the sections were fixed in freshly prepared paraformaldehyde (5 min) and washed extensively in 0.1 M phosphate buffered saline (pH 7.4; PBS) for 15 min. To enhance the permeability of the extracellular matrix, glycosaminoglycans were removed by incubating the sections with 1% hyaluronidase (testicular, type I-s, EC 3.2.1.35, Sigma, St Louis, MO) in PBS for 30 min at 37°C.

Non-specific staining was blocked by incubation of the sections with 10% normal horse serum (Col2-3/4M) in PBS with 1% bovine serum albumin (Sigma).

Sections were incubated over night with the Col2-3/4M antibody (1/800) against denatured type II collagen at 4°C in a humidified chamber. Biotin-labelled horse antimouse antibodies and goat antirabbit antibodies (DAKO, Glostrup, Denmark, 1/400) were used as secondary antibodies (1 h, room temperature). A biotin streptovidin detection system (Vectra elite kit, Vector, Burlingame, CA) was used according to the manufacturer's recommendations. Peroxidase was detected using tablets containing 10 mg 3′,3′diaminobenzidine (Sigma) dissolved in 15 ml PBS with 12 µl H_2O_2 (30%) for 7 min. After rinsing, sections were dehydrated and mounted with DPX (BDH, Poole, U.K.). Adjacent sections were stained with Toluidine blue to demonstrate glycosaminoglycans[14].

EVALUATION

The healing response of the meniscus was scored as none (no healing response), partial (at least 50% of the lesion was filled with repair tissue), or healed (at least 75% of the lesion was filled with repair tissue). The phenotype of the tissue in the empty access channels and the polymer implants was evaluated.

Macroscopically, all joint surfaces were evaluated directly after dissection for cartilage damage, which was graded on an ordinal scale as no degeneration (deg−), minor degeneration (discolored cartilage areas, deg+), mild degeneration (roughened cartilage surface, deg++), and presence of depressions or craters in the cartilage (crater)[16]. During microscopical evaluation the observer (TvT) was blinded to the treatment.

Microscopically, the femoral and tibial articular cartilage degeneration was evaluated on the basis of disruption of the normal architecture of the articular cartilage as could be observed on sections stained with Toluidine blue and hematoxyline-eosine. These degenerative articular changes were scored according to the Mankin grading system from normal structure (grade 0) to complete disorganization (grade 6), normal cells (grade 0) to hypocellularity (grade 3), normal Toluidine blue staining (instead of Safronine O staining, as used in the original Mankin score[17]) (grade 0) to no staining (grade 4) and an intact tide mark (grade 0) or a tidemark infiltrated with blood vessels (grade 1).

Toluidine blue staining was scored with an ordinal scale from no staining (−) to normal staining (++++) as in non-damaged articular cartilage.

Collagen type II degradation was evaluated on immunohistological sections. The presence of denatured collagen type II (positive staining Col2-3/4M) was assessed on an ordinal scale as absence of labeling (−), labeling in the cartilage surface layers (+), labeling down to the middle zone (++) and labeling down to the deeper zone of the cartilage (+++).

Data were statistically analysed by means of the *t*-test; *P*-values of less than 0.05 were considered significant.

Results

THREE MONTHS

Lesion healing and fibrocartilage formation

Data concerning the healing of the lesion are presented in Table I. In the ACI group, in three cases the lesion was healed, while in the ACE group no complete healing was observed. In the ACI group only one polymer contained fibrocartilage-like tissue (dog no. 1). The ACE group only showed fibrous tissue in the access channel.

Surface changes in the articular cartilage

Macroscopically, the lateral femoral condyle in the ACI group and in the ACE group seemed unaffected, while the aspect of the lateral tibial cartilage varied from absence of degeneration to craters in the cartilage. The core of degeneration was located directly opposite the access channel in the meniscus. Further away from this area the amount of cartilage damage decreased. No evident difference in macroscopic degeneration was found between the ACE and ACI group at this time point (Table I).

Microscopically, varying stages of cartilage degeneration were observed in the ACE and ACI group. Cases with less degeneration showed evident proteoglycan staining (dog nos. 4 and 9). Extensive fibrillation with clefts perpendicular to the surface was apparent in two cases in the ACI group compared with four in the ACE group [Fig. 2(a), (b)]. In these fibrillated areas, no flat superficial chondrocytes were seen and there was evident less proteoglycan staining in the pericellular matrix than in non-damaged areas of the cartilage. The only case with an intact smooth cartilage surface was seen in the ACI group. The Mankin degeneration tended to be higher in the ACE group than in the ACI group, however, this difference was not significant ($P=0.245$) (Table I).

All cases with fibrillated sites showed intense positive labeling of Col2-3/4M antibodies [Fig. 2(c)]. Cartilage cells in the surface layers were completely surrounded by labeled epitopes and labeling reached the deep zones of the articular cartilage. The degree of degradation in the different cases did not differ between the ACE and ACI group ($P=0.330$).

Besides evidence of fibrillation, four cases in the implant group showed loosening and swelling of the lower cartilage matrix, covered by an intact superficial layer (Table I). These areas of swelling showed minor PG staining and moderate collagen type II degradation [Fig. 3(a), (b)]. However, in all cases with swelling proteoglycan staining was present in the adjacent cartilage. Only a few round cells were observed but there were abundant empty cell lacunae in these swollen superficial areas [Fig. 3(c)]. This aspect of swelling was not visible around fibrillated areas.

SIX MONTHS

Lesion healing and fibrocartilage formation

In the ACI group, in two cases the lesion was healed, while in the ACE group again no complete healing was observed. In three cases in the ACI group the polymer contained fibrocartilage-like tissue (dog nos. 1, 3 and 4). Again, the ACE group showed only fibrous tissue in the access channel.

Surface changes in the articular cartilage

Macroscopically, again no cartilage degeneration of the lateral femoral condyles was found, while degeneration of the tibial articular cartilage was apparent. However, after

Table I
Tibial characteristics of the macroscopical and microscopical results in the individual cases in the four groups

Dog no.	Side	Phenotype tissue	Lesion healing	Macroscopy	Microscopy			
				Degeneration	Mankin score	TB staining	Collagen degradation	Swelling
(a) 3 months								
ACI								
1	R	Fibrocart	?	Crater	7	+	+	−
2	R	Fibrous	Healed	deg++	3	++	?	+
3	R	Fibrous	Partially	deg+	3	+++	−	+
4	R	Fibrous	Healed	deg−	0	+++	++	+
5	R	Fibrous	Healed	Crater	3	++	+++	−
6	R	Fibrous	Partially	deg+	1	+++	−	++
Average					2.8			
ACE								
7	L	Fibrous	Partially	Crater	7	++	+++	−
8	L	Fibrous	Partially	Crater	4	++	++	−
9	L	Fibrous	Partially	deg−	2	+++	−	−
10	L	Fibrous	Not healed	Crater	5	++	+++	−
11	L	Fibrous	Not healed	Crater	3	+++	+	+
12	L	Fibrous	Not healed	deg+	5	+++	+++	+
Average					4.3*			
(b) 6 months								
ACI								
1	L	Fibrocart	Partially	deg++	3	++	+++	−
2	L	Fibrous	Partially	deg+	1	++	+	−
3	L	Fibrocart	Partially	deg−	2	++	−	+
4	L	Fibrocart	Healed	deg−	2	+	++	−
5	L	Fibrous	Partially	deg−	1	++	−	+
6	L	Fibrous	Healed	deg−	1	++	++	−
Average					1.7			
ACE								
7	R	Fibrous	Partially	deg+	1	++	++	−
8	R	Fibrous	Not healed	deg+	1	++	++	−
9	R	Fibrous	Partially	Crater	3	++	++	−
10	R	Fibrous	Partially	deg+	0	+++	+	−
11	R	Fibrous	Partially	Crater	3	++	+++	−
12	R	Fibrous	Not healed	deg−	1	+++	−	−
Average					1.5*			

?, damaged sections; could not be evaluated.
ACI, access channel with implant; ACE, empty access channel. *Significant difference in average Mankin score between the ACE groups at 3 and 6 months.

this time, no craters were present in the articular cartilage in the ACI group.

Microscopically, damage was generally restricted to surface irregularities in the ACE and ACI group. Although the superficial cell layers were damaged and proteoglycan staining was decreased in these zones, extensive cell death had not occurred. The decrease in Mankin score between 3 and 6 months was only significant in the ACE group ($P<0.05$) (Table I).

Again, degradation of collagen type II was detected in the ACE and ACI group in areas of fibrillation in the upper layers. The degree of degradation in the different cases did not differ between these two groups ($P=0.330$). However, at this time, degradation was also especially apparent in these groups in areas where fibrillation had not occurred (Fig. 4). Labeling of Col2-3/4M in the upper layers of the articular cartilage was combined with an absence of proteoglycan staining, although chondrocytes still had a normal distribution and appearance [Fig. 4(a), (b)].

Only two cases in the ACI group showed loosening of the cartilage surface layers with swelling and empty cell lacunae. This aspect was not visible in the ACE group.

Discussion

In this study, we evaluated the presence and mechanism of articular cartilage degeneration after partial thickness access channels had been created between a lesion in the avascular area of the meniscus and the vascularized synovial lining, to determine the value of this technique as a alternative to partial meniscectomy. The ingrowth of tissue into a polymer implant and its effect on healing have been described elsewhere[4]. In short, after 3 months, the peripheral part of the polymer scaffold was invaded by fibrovascular tissue. After 6 months, fibrocartilage-like tissue was present in the polymer.

The authors already showed that an access channel between a meniscal lesion in the avascular area and the vascularized synovial lining leads to healing of the lesion[4]. If healing of the lesion can be achieved, partial meniscectomy will not be necessary. Various other reconstruction techniques have also been found to induce healing of lesions in the avascular part of the meniscus[18–25,26]. However, the consequences on articular cartilage degeneration have not been evaluated.

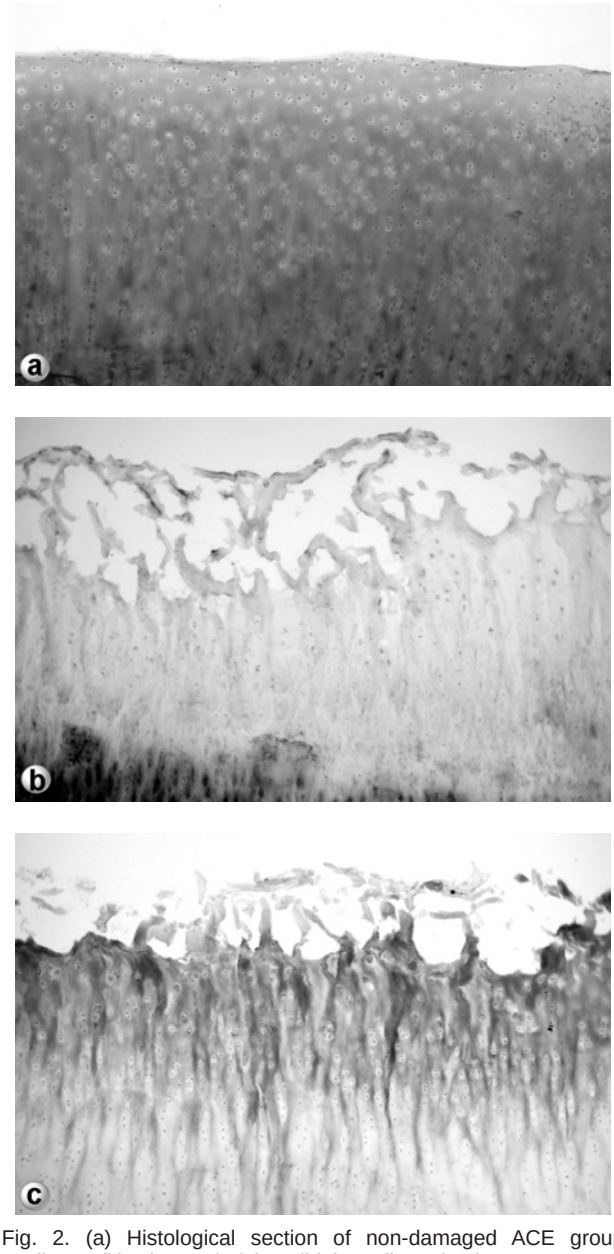

Fig. 2. (a) Histological section of non-damaged ACE group cartilage. (b) The underlying tibial cartilage in the ACE group, 3 months after surgery. Toluidine blue. Fibrillation of the tibial articular cartilage is apparent. This aspect was also visible in the ACI group. Magnification 100×. (c) Adjacent section with abundant Col2-3/4M antibody labeling reaching the deeper cartilage layers. Magnification 100×.

Meniscal reconstruction as applied in this study showed a high incidence of degenerative changes in the underlying cartilage, which consisted of fibrillation, loss of chondrocytes and decreased proteoglycan content. According to the literature, these changes also occur after partial meniscectomy[27–30]. The degeneration in the implant group mainly seemed to occur during a short phase after surgery, in which the implant had not yet become incorporated into the meniscus. After 3 months, the degenerative effect of the reconstruction might be less as the implant becomes better incorporated[4]. This assumption corresponds with the observation in the present study that the severity of cartilage degeneration did not increase from 3 to 6 months.

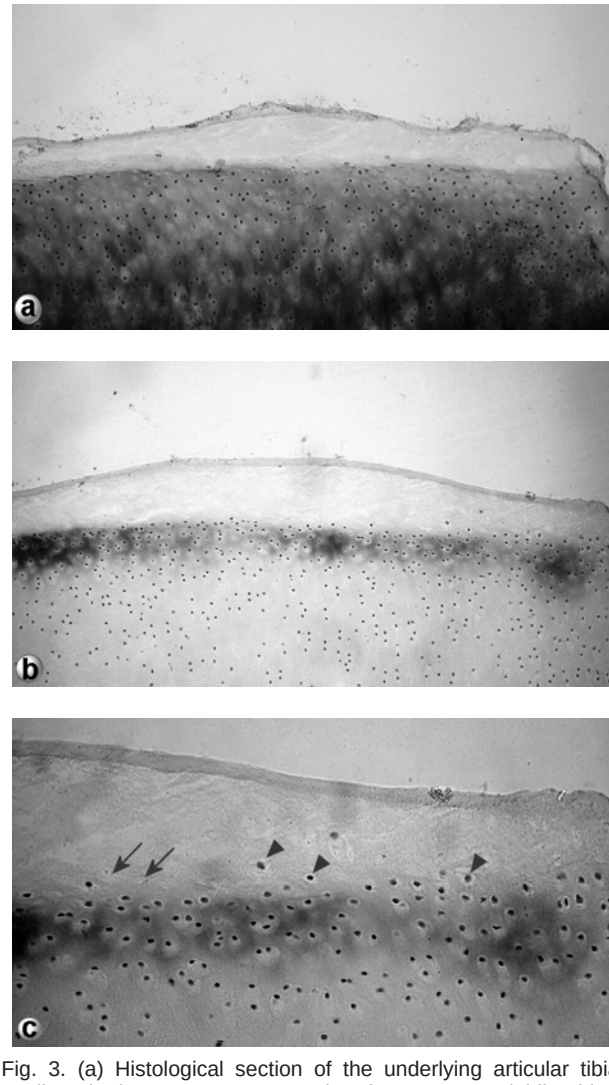

Fig. 3. (a) Histological section of the underlying articular tibial cartilage in the ACI group, 3 months after surgery. Toluidine blue. Loosening of the surface layer is visible with underlying swelling of the cartilage. Magnification 40×. (b) Adjacent section after labeling with Col2-3/4M. Only moderate degradation of collagen type II is visible. Magnification 40×. (c) Magnification of (b). Note the empty cell lacunae (arrows) and few remaining cells (arrow heads) in the transition zone. Magnification 200×.

Craters in the articular cartilage, observed after 3 months follow-up, were found after 6 months follow-up. Therefore, we speculate that in the longer-term, articular cartilage damage after reconstruction that leads to a healed meniscal lesion will be less severe than after partial meniscectomy. However, various ACLT models in dogs (according to Pond-Nuki) also did not show a progress of the OA over time[4]. This might imply that this animal model is not appropriate for evaluation of the consequences of meniscal reconstruction for the articular cartilage, or that the evaluation period is not long enough.

Six months after surgery, the ACE group even showed a significant decrease in Mankin score with less surface damage and more proteoglycan staining than after 3 months. This evident decrease in articular cartilage degeneration during longer follow-up, might suggest a reparative capacity of the canine articular cartilage. The reparative capacity in dogs was already suggested by

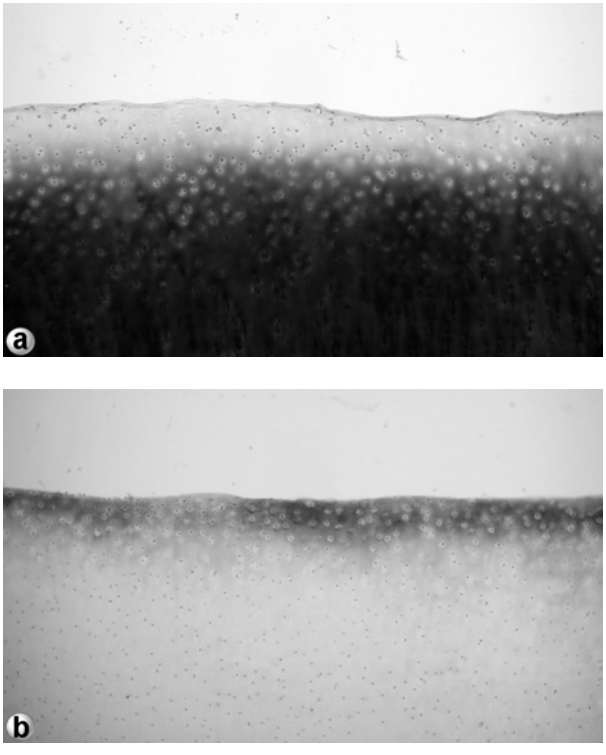

Fig. 4. (a) Histological section of cartilage in the ACE group with slightly decreased staining of Toluidine blue. Magnification 40×. (b) Adjacent section of (a). A non-fibrillated zone with positive labeling of Col2-3/4M 6 months after surgery. Magnification 100×.

Adams *et al.*, who reported an active synthetic response by the chondrocytes after anterior cruciate ligament transection (ACLT) resulting in hypertrophic cartilage repair[31]. In this way, the response of the canine cartilage seemed to differ from the that of human articular cartilage, in which loss of cartilage mass and proteoglycan synthesis is recognized as characteristic end stage OA[32]. Moreover, it should be emphasized that the axial loading pattern in the rather extended knees of man differs from the loading pattern in the flexed knees of dogs. When extrapolating these results to a human situation, all these factors should be taken into account.

ACLT models in dogs (according to Pond-Nuki)[5,6] and smaller animals[7,8] showed that weakening of the collagen network played an important role in the early morphological changes that lead to osteoarthritic cartilage. In these studies, it was hypothesized that mechanical influences after ACLT caused fibrillation, which in turn induced degradation of the underlying collagen network. If this were true, then collagen degradation would never be detected before fibrillation has occurred. In the present study, fibrillated cartilage areas always showed strong positive degradation antibody labeling. However, degradation was also observed in morphologically non-damaged areas of cartilage. Although the surface layers were still intact, the underlying cartilage matrix network may have become weakened, which might eventually lead to fibrillation of the articular cartilage.

Swollen areas in the underlying cartilage were especially apparent in cases with a polymer implant. In these areas, it can be considered that cell death had occurred in view of the decreased number of cells and the empty lacunae in the swollen regions. Also, proteoglycan staining was decreased; only moderate collagen degradation was visible in the underlying layers, while the upper surface layer was intact. This was not observed in the empty access channel group, in which the sharp edges of the meniscal defect might have caused direct damage to the articular cartilage. In the implant group, the cartilage was not exposed to the edges of the defect, but to a bare polymer surface. This might have caused different kind of strain and shear stresses in the underlying cartilage. The upper superficial layers were better protected against these stresses by the parallel oriented collagen fibers while the underlying matrix network, which might be more vulnerable, was weakened by these external forces[33].

In conclusion, creation of an access channel with or without porous polymer scaffold implantation led to degeneration of the articular cartilage. In this short-term experiment, creation of an access channel led to fibrillation of the underlying articular cartilage; in the implant group, the cartilage showed swollen areas with empty cell lacunae in the matrix beneath an intact surface layer. The damage did not increase between 3 and 6 months post-surgery. Degradation of collagen type II was observed in fibrillated areas, as shown in smaller animals, and also in non-fibrillated areas of the cartilage. We propose not only that degradation of the collagen type II network is the result of superficial mechanical cartilage damage, but also that weakening of the deeper collagen network might lead to morphological damage of the cartilage.

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