Autologous perichondral tissue for meniscal replacement

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Our aim was to examine the potential of autologous perichondral tissue to form a meniscal replacement. In 18 mature sheep we performed a complete medial meniscectomy. The animals were then divided into two groups: 12 had a meniscal replacement using strips of autologous perichondral tissue explanted from the lower rib (group G) and six (group C) served as a control group without a meniscal replacement. In all animals restriction from weight-bearing was achieved by means of transection and partial resection of tendo Achillis. Six animals (four from group G and two from group C) were each killed at 3, 6 and 12 months. The grafts and the underlying articular cartilage were removed and studied by gross macroscopic examination, light microscopy, SEM, polarised light examination, and by biomechanical tests.

In all the transplanted animals a new perichondral meniscus developed. After three months the transplants resembled normal menisci in size and thickness, while in the control animals only small rims of spontaneously grown tissue were seen. Microscopically, the perichondral menisci showed a normal orientation of collagen fibres and normal cellular characteristics, but in the central region, areas of calcification disturbed the regular tissue differentiation. Healing tissue in control animals lacked the normal fibre orientation and cellularity. SEM of perichondral menisci showed surface characteristics similar to those of normal sheep menisci without fissures and lacerations; the control specimens had these defects. The femoral and tibial cartilage in contact with the new menisci had normal surface characteristics apart from one animal with slight surface irregularities. Control animals showed superficial lesions after three months which increased at six to 12 months postoperatively. Microangiography of the newly grown tissue demonstrated a less intense vascularisation after three months when compared with normal menisci.

The failure stress and tensile modulus of perichondral menisci were significantly lower than those of normal contralateral menisci, and spontaneously regenerated tissue in meniscectomised animals had even lower values. There were no significant differences in values between newly grown perichondral menisci and spontaneously grown tissue.

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After subtotal or complete resection of the menisci, almost all patients develop arthritic changes.1-6 Meniscal replacement is the obvious solution if neither meniscal reconstruction nor minimal meniscectomy is possible.7-9 Experimental studies of meniscal transplantation have shown promising results for fresh or cryopreserved allografts.10-14 The problem of the transmission of viral diseases, however, has not been solved and there may also be difficulty with the optimal fit of the donor meniscus to the recipient. Meniscal replacement with an autologous tissue that has the potential to form a new meniscus may avoid these problems.

Experimental and clinical results of the treatment of deep hyaline cartilage defects with autologous perichondral grafting have shown regeneration of cartilage containing type-II collagen with encouraging shear modulus.15-20 This reflects the capacity of the relatively undifferentiated perichondrium to develop into a highly differentiated mesenchymal tissue. Our aim was to analyse the potential of this tissue to form a new meniscus.

Materials and Methods

We used 18 adult sheep (house strain) weighing 35 to 55 kg. Approval of the Institutional Review Board was
obtained for the experiments. After shaving and disinfection of the leg and thorax, the animals were anaesthetised first with intravenous ketamine (KetanestR 5%, 1 to 2 mg/kg/body-weight intravenously; Parke-Davies, Freiburg, Germany) and then with an intravenous infusion of ketamine (5 mg/kg/hour) in Ringer’s solution. Through a paracostal incision strips of perichondrium approximately 6 × 2 cm in size were elevated with a special punch from the underlying cartilage of the lower ribs near the sternum. The perichondral explants had a characteristic structure consisting of a fibrous layer, a proliferation zone and a transition zone (Fig. 1).

The medial compartment of the knee was exposed and the medial collateral ligament transected. In 12 animals (group G), strips of perichondrium were folded to form a new meniscal replacement with the former fibrous layer lying outwards. The tissue was individually adapted to the size of the original resected meniscus. The grafts were secured by non-resorbable sutures to the remaining transverse meniscal ligaments at the anterior and posterior horns and additionally by a running resorbable suture to the surrounding capsule. After fixation of the meniscal replacement, the medial collateral ligament was repaired and the joint closed in layers. A tenotomy and partial tenectomy of the calcaneal tendon were performed to allow restriction of weight-bearing in the immediate postoperative period. In the remaining six sheep (group C, control) the medial menisci were completely resected without replacement.

All animals received gentamicin (1.5 mg/kg/body-weight), mezlocillin (4 mg/kg/body-weight) and oxazillin (2 mg/kg/body-weight) intramuscularly before surgery and for three days thereafter. The original resected menisci from both groups were stored for comparison with the newly grown tissue.

Four animals of group G and two animals of group C were each killed at 3, 6 and 12 months postoperatively. All specimens of both groups were evaluated macroscopically, under polarised light, histologically, ultrastructurally and biomechanically. We performed microangiography in one animal chosen randomly from each group and at each time interval. Macroscopic evaluation involved the assessment of the shape and measurement of the horizontal size of the newly grown tissue. For comparative purposes, we also harvested 12 medial menisci from the contralateral knee. Each specimen was traced on to graph paper and the area of the specimen computed. For micromorphological evaluation of cartilage lesions, the corresponding femoral and tibial cartilage was removed and assessment of the femoral and tibial articular surface performed according to the classification of Outerbridge.

**Histological examination.** Each specimen was divided into three parts (Fig. 2) for light microscopy, examination by polarised light and SEM. For light microscopy, specimens were fixed in 4% paraformaldehyde in phosphate buffer, dehydrated in graded ethanol and embedded in paraffin. Osteocartilaginous specimens were decalcified (Dekalci-ferR; Histo-Products, San Diego, California). Sections, 6 μm thick, were stained with haematoxylin and eosin and Goldner’s trichrome stain. For examination under polarised light, specimens were prepared using the Lierse modification of the Spalteholz method. Specimens for SEM were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer,
postfixed in OsO₄, dehydrated in graded alcohols, dried, coated with gold palladium and examined by a Zeiss DSM 940 operating microscope with accelerating voltages between 10 and 25 kV.

**Biomechanical analysis.** This included measurement of the maximal failure stress and the tensile modulus of the newly formed meniscal replacement and comparison with normal medial menisci and the spontaneously regenerated meniscal substitutes. The removed grafts and the contralateral medial menisci were stored at –20°C until measurement was carried out. After thawing, the specimens designated for biomechanical tests (Fig. 2) were cut horizontally to a thickness of 1 mm with a cryotome. With a special punch dumb-bell-shaped specimens were made and fixed with cyanoacrylate between the specially designed clamps of a tensiometer. The free length of the specimen was 2 mm and the cross-sectional area at the smallest part designated for the failure stress test measured 1 mm². The measurement of failure stress was tested at 20°C with a displacement rate of 3 mm/min using a uniaxial testing machine (Zwick 7025-3; Ulm, Germany). Specimens were kept moist throughout the test period with isotonic saline. Before testing, a preload of 0.25 N was applied for one minute. Cross-head displacement and tensile force were recorded on load-deformation diagrams during testing to failure. For statistical evaluation, we used the non-parametrical Kruskal-Wallis and Mann-Whitney U tests for small numbers of specimens.

**Microangiography.** One animal of each group chosen at random at each time interval was anaesthetised and heparin (Heparin-Natrium, 20 000 IU; Ratiopharm, Ulm, Germany) given intravenously. The femoral artery of the operated leg was cannulated and perfused with 500 ml of dextran 40 solution. After killing the animal, the hind leg was disarticulated at the hip and perfused with 500 ml of a radiopaque 1:4 suspension containing barium sulphate (Micropaque R; Nicholas, Sulzbach, Germany) in Ringer’s solution. Perfusion was continued with a 1:2 suspension, with a pressure of 200 cm H₂O for 12 hours. After harvesting the specimens soft radiographs were made (MammomatR, Siemens; Micropuls NIF 100 film; DuPont de Nemours, Frankfurt, Germany). For comparative purposes microangiography was also performed on two non-operated contralateral legs.

**Results**

No infections or other postoperative complications were seen. All the animals had a limp for at least four weeks postoperatively. At the area of resection of the calcaneal tendon the scar tissue was thickened. The enlarged cross-section decreased with time. After six to eight weeks all sheep could walk normally.

**Macroscopic findings**

Group G. Independent of the time interval all perichondral menisci had an almost normal meniscal size in the horizontal plane. None was smaller than 80% of the normal and none showed evidence of ruptures. The height of the newly-generated menisci was normal in all specimens. After three months, three animals had a newly-formed perichondral meniscus which had the same horizontal size as a normal meniscus. In the remaining animal in this group the size was 90% of the normal meniscus. After six months three of the four new menisci were similar to normal menisci and at 12 months half of the grafted menisci resembled normal menisci in shape and size (Fig. 3a). The rest had a horizontal size of 80% of the normal meniscus.

Group C. At all the time intervals regenerated tissue with a meniscoid shape, but thinner and smoother than normal menisci, was seen (Fig. 3b). The size ranged from 25% to 40% of normal menisci.

**Articular surface.** In group G one animal had a superficial surface irregularity (after 12 months). Most of the control animals showed chondromalacia of the femoral and tibial articular surface, which became worse with time.
**Histological findings**

*Original menisci.* These showed the typical meniscal structure, with collagen fibre bundles predominantly of the circular type and radiating fibres mostly confined to the femoral articular surface.

*Group G.* After three months the perichondral transplants had a meniscus-like configuration in the triangular cross-section and horizontal plane. Near the surface fibrochondrocytes were seen increasing from the free edge to the periphery. The orientation of the collagen fibre bundles was similar to that of a normal meniscus, but the perichondral menisci had blood vessels at the edge. A superficial layer of synovial cells, two to three cell rows thick, was present with hypertrophy in the periphery. All perichondral menisci had central areas of a cellular differentiation similar to hyaline-like cartilage and were associated with areas of central calcification.

*Group C.* In all specimens loose collagen fibre bundles and fibrochondrocytes were seen, but were not orientated.

**Polarised light examination**

*Original menisci.* These showed the well-known arcade-like orientation of collagen fibres with a circular orientation at the base and a radial orientation towards the free edge with a transition zone between (Fig. 4a).

*Group G.* Perichondral menisci had a fibre orientation similar to that of normal menisci, but in the central calcified areas no fibres could be identified (Fig. 4b).

*Group C.* These had a loose circular fibre orientation at the periphery and areas with no orientation. No arcade-like formation was visible at any time.

**Scanning electron microscopy**

*Original menisci.* These had a regular surface structure with crossing bundles (Fig. 5a).
**Group G.** Perichondral menisci had a structure similar to the fibre bundle of original menisci, but with a more irregular orientation. No fissures, ruptures or other damage could be seen (Fig. 5b).

**Group C.** The meniscoid tissue was small, smooth and thin with no regular orientation of the fibre bundles. There was loose connective tissue with fissures in the central parts.

**Articular cartilage.** In all but one of the femoral and tibial specimens of group G the characteristics of the articular surface were normal. In one animal cartilage lesions (with fissures, fibrillation, and demasking of the fibres) were seen 12 months after meniscal replacement at the femur and tibial plateau. In group C the femoral specimens showed less destruction than those of the tibial plateau but lesions were visible after three months and increased with time. At three months superficial fibrillations were seen which progressed to deep fissures with demasked fibres by 12 months.

**Microangiography**

**Original menisci.** The normal pattern of vascularisation was seen with blood vessels only in the peripheral third (Fig. 6a).

**Group G.** There was less intense vascularisation in the anterior and posterior horns compared with normal specimens at three months. The vascularisation improved after six months, but even after 12 months all parts of the perichondral meniscus had less intense vascularisation than the normal meniscus (Fig. 6b).

**Group C.** All specimens had some basal blood vessels but distinction between capsular and meniscal vessels was impossible.

**Biomechanical results.** Compared with the original menisci, the failure stress and the tensile modulus of perichondral menisci were significantly lower (Fig. 7). Spontaneously regenerated tissue had significantly smaller values than the
Discussion

Kohn et al\textsuperscript{23} and Kohn and Wirth\textsuperscript{24} examined the potential of the infrapatellar fat pad to rebuild a meniscus in the sheep knee. Because of the low histological and biomechanical quality of this tissue it was not recommended. In further experiments they used autologous fascia lata and patellar tendon grafts\textsuperscript{9} which gave more promising results.

Our results showed that articular cartilage below and beyond the perichondral meniscus had a normal appearance in all but one animal after 12 months which corroborated the finding that perichondrial menisci can, in principle, protect the articular surface from degenerative changes. Structurally, the regrown perichondral menisci resembled normal sheep menisci. In contrast to Kohn et al\textsuperscript{9} we found no tears in the newly-grown replacements probably because of the structure of the original perichondrial tissue.

Perichondral menisci had areas of central calcification in the pars intermedia which may be related to their subjection to greater axial loads. This may stimulate differentiation of the pars intermedia which may be related to their subjection to that of the original perichondrium with the fibres lying perpendicular to the applied tensile force. Our specimens were mostly taken from calcified areas where there was not a regular fibre orientation. This assumption is supported by the fact that in some specimens, without calcification, a distinct but non-significant increase in failure stress was found.

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References

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