Repair of sheep articular cartilage defects with a rabbit costal perichondrial graft

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A xenograft of costal rabbit perichondrium was transplanted to an articular cartilage defect in a sheep knee. After 12 weeks, cartilage was formed with increased calcification of the basal layer and a mean of 74 percent collagen type II. The synovium did not show any infiltration, indicating the absence of any immunologic reaction.

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Articular cartilage defects heal with fibrous or fibrocartilaginous tissue (Mitchell and Sheppard 1976, Furukuwa et al. 1980, Salter et al. 1980) of poor mechanical quality (Nelson et al. 1988). With an autologous perichondrial graft, these lesions can be repaired with a tissue that has the histologic and biochemical characteristics of articular cartilage (Engkvist and Ohlsen 1979, Woo et al. 1987, Amiel et al. 1988 and Homminga et al. 1989). Heterologous tissue evokes an immunologic reaction causing rejection of the graft. In contrast to isolated heterologous chondrocytes, which are immunogenic (Bentley 1971), allografts of intact joint cartilage do not allow contact between antibodies and chondrocytes (Peacock 1960, Langer and Gross 1974). Allogenic articular chondrocytes embedded in a collagen gel likewise do not evoke an immunologic reaction (Wakitani et al. 1989).

Little is known about the antigenicity of perichondrium. To assess the possibility of resurfacing joints, it is important to know whether or not tissue derived from a perichondrial xenograft resembles articular cartilage. Perichondrium taken from the rib contains mainly collagen type I (Amiel et al. 1988). The same applies to typical fibrocartilage, such as the semilunar meniscus of the knee (Eyre and Muir 1975) and spontaneous repair tissue in cartilage defects (Mitchell and Sheppard 1976 and Furukuwa et al. 1980). In hyaline cartilage, mainly type II collagen is present, which determines the physical properties of the tissue.

In this animal experiment, we investigated whether or not xenografts of costal perichondrium can form hyaline cartilage.

Material and methods

Costal perichondrium was dissected from the cartilaginous part of one of the lower ribs of 10 male rabbits (crossbred Flamish giants), weighing 3.5–5.7 kg. The graft was kept in normal saline. Anesthesia was given using i.v. ketamine hydrochloride (100 mg/kg) and diazepam (8 mg/kg). Ten adult female sheep (Texel breed), weighing 54–61 kg, received the grafts within 1 hour after dissection. Under Pentothal anesthesia the sheep were intubated and then kept under sedation with Fluothane (halothane). The knee was opened with a medial parapatellar incision. A full thickness cartilage defect, measuring 5 × 10 mm, was created in the weight-bearing area of the medial femoral condyle down to the subchondral bone until point hemorrhages were seen. Tissucol®, a human fibrin glue, was used for initial fixation of the graft. The rabbit perichondrium (Figure 1), with a mean thickness of 230 (*SD* 20) μm, was placed in the defect with the chondral side facing the joint. The knee was immobilized with a unilateral external fixation device to prevent the graft from loosening (Widenfalk et al. 1986). A tenotomy of the achilles tendon was performed to prevent weight bearing in the early postoperative period. Two weeks later, the external fixation device was removed and the operated on leg was placed in a motor unit 8 hours a day for 14 days. In this way, continuous passive motion was applied, stimulating chondrogenesis (Salter et al. 1980). After this period of time, the sheep were allowed to move about freely in the meadow. Three months after the transplantations, the results were evaluated.

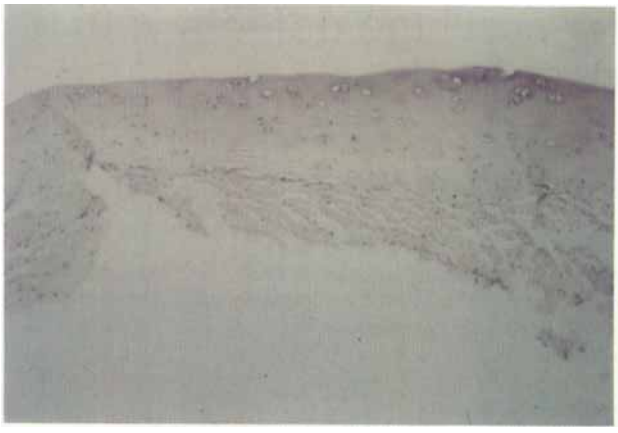

Figure 1. Histology of rabbit perichondrium showing three different layers. H&E, ×144.

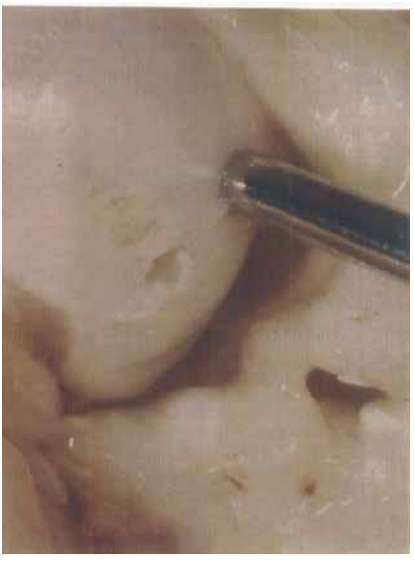

Figure 2. Macroscopic view of the grafted area. A biopsy is taken from it; another from the medial femoral condyle serves as a control.

Walking pattern. The animals were observed during walking to determine whether or not they limped.

Gross observation of the joint, synovium and perichondrial graft. The animals were killed and the operated on knee was opened. The joint was inspected for intraarticular fluid and the synovium for swelling and increased vascular injection. The articular surface was inspected together with the perichondrial graft.

Histology of graft and control. Core biopsies, 2 mm in diameter, were taken from the graft and another site of the same femoral condyle (Figure 2). The specimens were fixed in neutral formalin, embedded in paraffin, sectioned at 10 µm, and stained with hematoxylin and eosin (H&E), Alcian Blue PAS, and according to Erös (Romeis, 1968). The tissue derived from the perichondrial graft was evaluated in terms of graft thickness, cellular form, organization (H&E), and matrix production (Alcian Blue PAS). Erös' staining was used to visualize calcium.

Histology of the synovium of the knee. With a surgical knife, a piece of synovium measuring approximately 1 × 1 mm was cut from the medial side of the operated on knee. These specimens were stained with hematoxylin and eosin and studied under a light microscope. Special attention was paid to the presence or absence of monocyte or granulocyte infiltration—with their presence being a sign of immunologic rejection of the graft.

Biochemistry—collagen typing of graft and control cartilage. Biopsies were taken from the perichondrial graft and from another area of the same femoral condyle for quantitative analysis of the collagen type according to the method of O'Driscoll et al. (1985) using sodium dodecyl sulfate polyacrylamide gel electrophoresis of cyanogen bromide generated peptides. Collagen I from rat tail tendon and collagen II from human articular cartilage, isolated as described by Kuijer et al. (1985), were used as standards. Special attention was paid to the ratio collagen I/collagen II in the newly formed tissue after 3 months.

Results

One knee developed septic arthritis. After 3 months, 9 of the sheep had a normal walking pattern and a knee without synovial swelling. No intraarticular fluid was detected. All the knees had a smooth articular surface. In all the cases the cartilage defect was completely filled up with a tissue resembling articular cartilage to the level of the surrounding joint surface. Slight variability was seen in relation to color, height, and junction between the graft and underlying subchondral bone (Figure 3).

Biopsies taken from the synovium showed normal synovial lining cells without infiltration of granulocytes or monocytes.

In the specimens taken from the graft, the cells exhibited the characteristic lacunae of chondrocytes. The mean thickness of the grafts was 1,150 (*210*) µm compared with a mean thickness of the cartilage of the

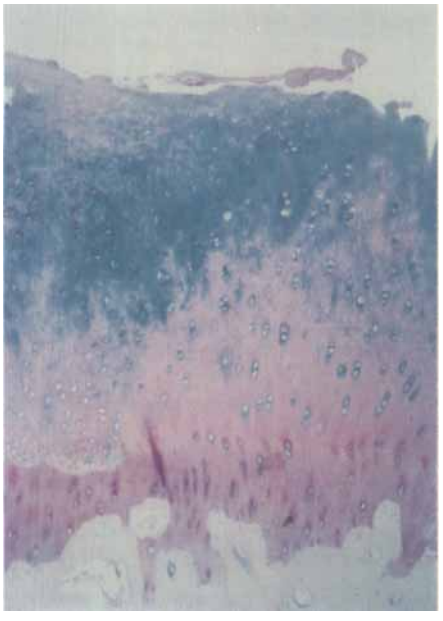

Figure 3. Histology of cartilage formed out of a xenograft of perichondrium 3 months after transplantation. Alcian Blue PAS, ×144.

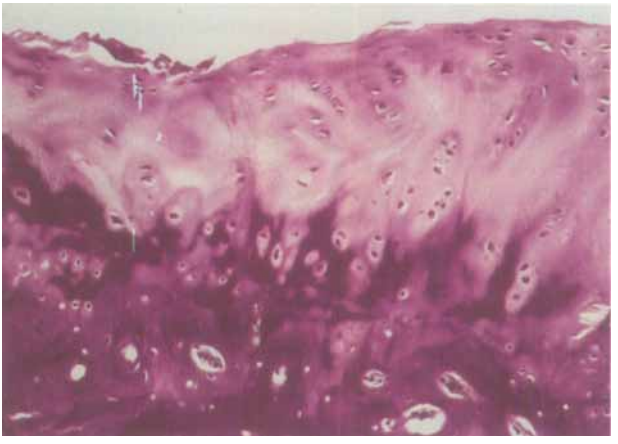

Figure 4. Specimen taken from the graft 3 months after heterologous transplantation of perichondrium. Cartilage with normal-shaped chondrocytes. In the basal layer increased staining is visible indicating a high calcium concentration. Erös' staining, ×144.

femoral condyle of 1,080 (*250*) µm. The repair tissue showed some variability in cell number: in the central part of the biopsy, the mean cell number in an area of 0.5 × 0.05 was 880 (*182*) per mm^2 as compared with a cell number of 965 (*120*) in a similar area in the cartilage of the same condyle. The organization of the tissue appeared very similar to the articular cartilage taken from the control site of the femoral condyle. Normal staining properties in the matrix (Alcian Blue) indicated the presence of proteoglycans as in hyaline cartilage. The junction between the newly formed cartilage and underlying bone showed no clefts and was uninterrupted. The calcium staining according to Erös showed an intense staining of the basal layer compared with the control, indicating increased mineralization of this layer (Figure 4).

Biochemical analysis of the collagen types present in the newly formed cartilage revealed the presence of 74 ± 14 percent collagen II (collagen I + collagen II = 100 percent). In comparison, rabbit perichondrium contained 100 percent collagen I and sheep articular cartilage from the femoral condyle of the same knee 94 ± 3 percent collagen II. This percentage of type II collagen is higher but statistically not different from the percentage found by Amiel et al. (1980) in autologous perichondrial grafts in the rabbit after 3 months.

Discussion

Allografts of articular cartilage evoke little immunologic response. In this experiment a xenograft of perichondrium appeared to have a very low antigenicity, as was demonstrated by the absence of lymphocyte infiltration of the synovium of the knee and the subchondral bone below the graft. Moreover, the difference in genotype was not found to influence the formation of repair tissue with the histologic characteristics of articular cartilage. The increased presence of calcium in the basal layer accords with the findings of Bab et al. (1982), who found calcifications associated with cartilaginous-forming cells and extracellular matrix vesicles after transplantation of free perichondrial grafts into rabbit articular cartilage. Homminga et al. (1990) found increased mineralization of an autogenic graft after clinical use of perichondral grafting of chondral lesions in the knee. It is unknown whether this increased mineralization will influence the durability of the neocartilage. Previous reports by Amiel et al. (1988) described a gradual increase in the type II collagen found in the perichondrial grafts: from 62 percent after 12 weeks to 82 percent after 1 year. The mean percentage of collagen type II found in the grafts of our experiment after 3 months agrees with these figures. Perichondrial xenografting seems in principle to be a suitable technique for repairing articular cartilage defects, although long-term results still have to be assessed.

References

Amiel D, Coutts R D, Harwood F L, Ishizue K K, Kleiner J B. The chondrogenesis of rib perichondrial grafts for repair of full thickness articular cartilage defects in a rabbit model: A one year postoperative assessment. *Connect Tissue Res* 1988; 18 (1): 27–39.

Bab I, Sela J, Stein H. Transplantation of free perichondrial grafts into rabbit articular cartilage is associated with matrix vesicle calcification. *Acta Anat* (Basel) 1982; 113 (1): 53–60.

Bentley G, Greer R B. Homotransplantation of isolated epiphyseal and articular cartilage chondrocytes into joint surfaces of rabbits. *Nature* 1971; 230: 385–8.

Engkvist O, Ohlsen L. Reconstruction of articular cartilage with free autologous perichondrial grafts. An experimental study in rabbits. *Scand J Plast Reconstr Surg* 1979; 13 (2): 269–74.

Eyre D R, Muir H. The distribution of different molecular species of collagen in fibrous, elastic and hyaline cartilages of the pig. *Biochem J* 1975; 151 (3): 595–602.

Furukawa T, Eyre D R, Koide S, Glimcher M J. Biochemical studies on repair cartilage resurfacing experimental defects in the rabbit knee. *J Bone Joint Surg* (Am) 1980; 62 (1): 79–89.

Homminga G N, van der Linden T J, Terwindt Rouwenhorst E A, Drukker J. Repair of articular defects by perichondrial grafts. Experiments in the rabbit. *Acta Orthop Scand* 1989; 60 (3): 326–9.

Homminga G N, Bulstra S K, Bouwmeester P S, van der Linden A J. Perichondral grafting for cartilage lesions of the knee. *J Bone Joint Surg* (Br) 1990; 72 (6): 1003–7.

Kuijer R, van de Stadt R J, de Koning M H, van der Korst J K. Influence of constituents of proteoglycans on type II collagen fibrillogenesis. *Coll Relat Res* 1985; 5 (5): 379–91.

Langer F, Gross A E. Immunogenicity of allograft articular cartilage. *J Bone Joint Surg* (Am) 1974; 56 (2): 297–304.

Mitchell N, Shepard N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. *J Bone Joint Surg* (Am) 1976; 58 (2): 230–3.

Nelson B H, Anderson D D, Brand R A, Brown T D. Effect of osteochondral defects on articular cartilage. Contact pressures studied in dog knees. *Acta Orthop Scand* 1988; 59 (5): 574–9.

O'Driscoll S W, Salter R B, Keeley F W. A method for quantitative analysis of ratios of types I and II collagen in small samples of articular cartilage (published erratum appears in *Anal Biochem* 1988; 174 (1): 360). *Anal Biochem* 1985; 145 (2): 277–85.

Peacock E E, Weeks P M, Petty J M. Some studies on the antogenicity of cartilage. *Ann New York Acad Sci* 1960; 87: 175–83.

Romeis B. *Mikroscopische Technik*. Oldenbourg Verlag, München, Wien 1968: 403.

Salter R B, Simmonds D F, Malcolm B W, Rumble E J, MacMichael D, Clements N D. The biological effect of continuous passive motion on the healing of full thickness defects in articular cartilage. An experimental investigation in the rabbit. *J Bone Joint Surg* (Am) 1980; 62 (8): 1232–51.

Wakitani S, Kimura T, Hirooka A, Ochi T, Yoneda M, Yasui N, Owaki H, Ono K. Repair of rabbit articular surfaces with allograft chondrocytes embedded in collagen gel. *J Bone Joint Surg* (Br) 1989; 71 (1): 74–80.

Widenfalk B, Engkvist O, Ohlsen L, Segerström K. Perichondrial arthroplasty using fibrin glue and early mobilization. An experimental study. *Scand J Plast Reconstr Surg* 1986; 20 (3): 251–8.

Woo S L, Kwan M K, Lee T Q, Field F P, Kleiner J B, Coutts R D. Perichondrial autograft for articular cartilage. Shear modulus of neocartilage studied in rabbits. *Acta Orthop Scand* 1987; 58 (5): 510–5.