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Autologous rib perichondrial grafts in experimentally induced osteochondral lesions in the sheep-knee joint: morphological results

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Summary. The purpose of the present study was to examine the fate of autologous perichondrial grafts after transplantation into cartilage lesions in weight-bearing joints. Osteochondral lesions were made in the articular surface of knee joints in 36 sheep. The defects were filled with autologous rib perichondrial grafts which were secured by either collagen sponges (12 animals) or fibrin glue (12 animals). Defects without perichondrial grafts served as controls (12 animals). Following 1 week of immobilization of the operated leg, the plaster was removed and the animals were allowed to move freely. Animals were sacrificed after 4, 8, 12 and 16 weeks. The grafts were removed and investigated histologically. In contrast to weight-bearing areas and control defects, hyaline-like cartilage formation was seen in non-weight-bearing areas after 4 weeks. This newly formed cartilage revealed strong metachromasia following staining with acidic toluidine blue and reacted positively with periodic acid-Schiff, indicating de novo synthesis of proteoglycans and glycoproteins. Scanning electron microscopy and examinations with polarized light confirmed a hyaline cartilage-like architecture for the surface area as well as for the fibre orientation of the whole graft. Enzyme histochemistry for alkaline and acid phosphatase activity showed positive reactivity only at the base of the transplants.

Key words: Perichondrium – Cartilage defects – Cartilage transplantation – Fibrin glue – Collagen sponge

Introduction

The results of conservative or operative treatment in patients with deep osteochondral lesions, especially in weight-bearing joints, are still unsatisfactory. The defects which follow trauma or osteochondritic processes with loose bodies are replaced by scar tissue; osteoarthritis usually develops within a few years (Bentley 1978; Calandruccio and Gilmer 1962; Johnson 1986; Meachim and Roberts 1971; Mitchell and Shepard 1980; Nelson et al. 1988).

The chondrogenic potential of the perichondrium has been evaluated by several investigators who have demonstrated that de novo chondrogenesis can be observed after transplantation (Amiel et al. 1985a, b; Engkvist 1979; Engkvist and Ohlsen 1979; Engkvist et al. 1979; Homminga et al. 1989, 1990; Kon 1981; Skoog et al. 1972; Woo et al. 1987). Biochemical and biomechanical analysis of the newly formed cartilage have revealed biological similarity between the grafts and hyaline cartilage (Amiel et al. 1985a, b, 1988; Homminga et al. 1989; Maruyama 1979; Woo et al. 1987). According to these results perichondrial grafting seems to be successful in small animals, such as rabbits, but for clinical application these data have to be confirmed in joints of large animals which have biomechanical similarity to human joints.

It was the aim of this study to investigate the regenerative potential of grafted perichondrium in weight-bearing joints in sheep. Furthermore, some specific problems of fixation of the grafts as well as elucidating the influence of weight-bearing had to be investigated prior to clinical application.

Materials and methods

Thirty-six 5-month-old sheep, Awadassi/Ostfriesen strain, weighing 35–55 kg (from the Department of Animal Research Technion, Haifa, Israel) were used. The animals were kept under standard conditions with free access to food and water, but were fasted 24 h before the operation. Animals were anaesthetized with 3–4 ml sodium pentobarbital (60 mg/ml), shaved, and lateral X-rays of the knee were taken in flexed and extended joint positions. The legs were prepared with Betadine (povidone-iodine), and draped in a sterile fashion prior to the operation. Perichondrial grafts were obtained from the lower ribs using a special punch (measuring 9 mm in diameter), and were kept under sterile conditions in 0.9% saline solution at room temperature. Thereafter, the hindleg was operated via a medial parapatellar incision. The muscles were dissected by layers, the knee joint was opened and the patella was dislocated laterally exposing the medial femoral condyle and the patellar groove.

Two full-thickness holes (7 mm in diameter) were drilled

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through the articular cartilage of the medial femoral condyle, which represented a weight-bearing area of the knee joint, until bleeding was encountered from the subchondral bone (Fig. 1 A). Two drill-holes were performed in the most inferior medial and lateral patellar groove, which represented non-weight-bearing areas. The perichondrial grafts were transferred to the drill-holes and were placed so that their cartilaginous surface faced the joint cavity.

There were two experimental groups. In group A (12 animals) xenologous lyophylized human fibrin glue (Tissucol; Immuno, Vienna, Austria) was used for fixation of the graft into the four defects (two in the non-weight-bearing and two in the weight-bearing area). In group B (12 animals), thin xenogenous bovine collagen sponges (Helitrex; American Biomaterials, Princeton, N.J., USA) were used for graft fixation. Group C (12 animals) was a control group with drill-holes but without any transplantation. These drill-holes were filled with either fibrin-glue (4 animals) or collagen sponges alone (4 animals) or were kept without any filling (4 animals). Finally, the joints were closed by layers using resorbable sutures, and the skin closed with non-resorbable sutures. All animals received procaine-penicillin (2 g) and streptomycin (1 g) intramuscularly prior to surgery and for 3 days thereafter. Immediately following surgery the operated legs were immobilized in plaster of Paris for 1 week. Animals were allowed to move freely within their cages, and after removal of the cast, they were returned to active motion under farm conditions.

Three animals in each group (A, B, C) were sacrificed after 4, 8, 12 and 16 weeks postoperatively. Lateral X-rays of the knee joints were taken in flexed and extended joint positions once again.

The repair process was evaluated macroscopically, histologically, histochemically, and ultrastructurally. Macroscopic evaluation involved assessment of the status of the articular surface (smooth or disrupted), the degree of the adherence of the grafts to adjacent cartilage and the surface level of the newly formed tissue. For microscopic evaluation, the defects and engrafted areas were chiseled out together with a rim of original intact surrounding cartilage and underlying subchondral bone. Each specimen was then divided into four parts.

One part, for light-microscopic analysis, was fixed in 4% paraformaldehyde in phosphate buffer, pH 7.2, at room temperature, decalcified in 10% ethylenediaminotetraacetic acid (EDTA) buffered with TRIS-HCl, pH 7.2, dehydrated in graded ethanols and embedded in Paraplast (Fisher, Pa., USA). Sections, 6 μm thick, were stained with haematoxylin and eosin, with toluidine blue at pH 2.0 and with the periodic acid-Schiff reaction (PAS) for demonstration of acidic and neutral polysaccharides (Silbermann and Frommer 1974; Silbermann et al. 1977).

Another part designated for scanning electron microscopy (SEM) was fixed in 5% glutaraldehyde in 0.1 M cacodylate buffer, postfixed in OsO_4, dehydrated in graded alcohols, dried, coated with gold-palladium and examined with a Zeiss Novoscan 30 Scanning electron microscope operating at 15 kV.

Those specimens for enzyme histochemistry were quenched in liquid nitrogen (−186° C) and stored at −70° C. Sections 8 μm thick were cut in a cryostat (−25° C) and were subsequently reacted for alkaline phosphatase and acidic phosphatase using the azo dye-coupling method (Bancroft and Stevens 1977).

The parts of the specimens for polarized light examination were prepared using the Lierse modification of Spalteholz method (Lierse 1960).

Results

Throughout the experimental period neither signs of infection nor limping were encountered. No contractures of the operated joints were observed. In 14 animals 5–10 ml of clear joint effusion were found at either 4 weeks or 8 weeks after transplantation, particularly in sheep treated with collagen sponges.

Macroscopically, in most of the non-weight-bearing areas in animals of group A and B, the surface level of the newly formed tissue reached the level of the surrounding cartilage by 4 weeks. However, the new tissue in weight-bearing areas appeared depressed and its surface was disrupted (Fig. 1 B). Of 48 transplanted defects in the non-weight-bearing area only 2 grafts that were fixed either with collagen sponges (1, group B, 8 weeks postoperatively) or fibrin glue (1, Group A, 12 weeks postoperatively) were found as loose bodies.

In group C (control defects) drill-holes filled with either fibrin glue or with collagen sponge alone or not treated at all revealed only incomplete filling with scar-like tissue.

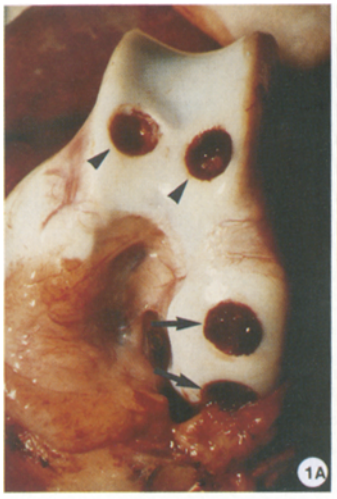
Microscopic examination of groups A and B of the non-weight-bearing area out of 48 transplanted grafts showed in 40 [20 grafts fixed with fibrin glue (=group A), 20 grafts fixed with collagen sponge (=group B)] the development of hyaline-like cartilage. Six defects [3 fixed with fibrin glue (=group A), 3 fixed with collagen sponge (=group B)] demonstrated differentiation into fibrocartilage only. In drill-holes of transplanted joints in which loose bodies were found only scar tissue formation was observed.

The repair process in specimens of the non-weight-bearing area of both groups (A and B) followed a typical course of graft integration. Specimens 4 and 8 weeks after transplantation showed remnants of the donor fibrous tissue (the perichondrium) at the base of the graft close to the subchondrial bone (Fig. 2 B).

After 8 and 12 weeks a few healing defects of group B exhibited small foci of cartilage necrosis, corresponding to the transition zone between the original perichondrial grafts and the new cartilage. Concomitantly, at the deeper portions of the transplants a slight lymphocytic infiltrate was encountered in transplants of groups A and B in the non-weight-bearing area. In defects in

Fig. 1. **A** Sheep knee joint with two drill-holes, 7 mm in diameter, in the non-weight-bearing area (patellar groove, *arrowheads*) and two drill-holes in the weight-bearing area at the femoral condyle (*arrows*). All defects show bleeding from the subchondral bone indicating a full-thickness defect. **B** Macroscopic findings in drill-holes in the non-weight-bearing and the weight-bearing area; 4 weeks after transplantation grafted defects in the non-weight-bearing area are totally filled up with a glistening material similar to the surrounding normal articular cartilage, whereas in the weight-bearing area defects are only filled up with a greyish tissue differing from the normal surrounding cartilage

Fig. 2. **A** Control defect without perichondrial graft 4 weeks after drilling: scar tissue without any cartilaginous differentiation fills the defect. Haematoxylin and eosin (H&E), ×64. **B** Perichondrial graft 4 weeks after transplantation into a drill-hole and fixation with fibrin glue in the non-weight-bearing area: hyaline-like cartilage differentiation can be seen in the superficial part of the graft, whereas at the base of the transplant (→) the original fibrous part of the perichondrium is still visible. H&E, ×120. **C** Perichondrial graft from a weight-bearing area 8 weeks after transplantation. There is depression of the surface and mainly fibrous tissue with small areas of fibrous cartilage filling the defect. H&E, ×120. **D** A perichondrial graft 16 weeks after transplantation into a drill-hole of the non-weight-bearing area. Hyaline-like cartilage with a higher cell density as normal cartilage is visible. At the joint surface cells are more horizontally orientated, whereas in the middle and deep zone cells begin to form a more perpendicular cell orientation. Masson-Goldner, ×120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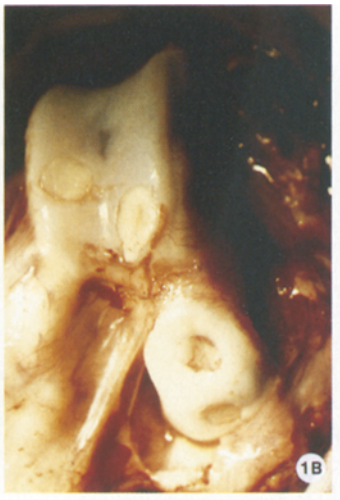
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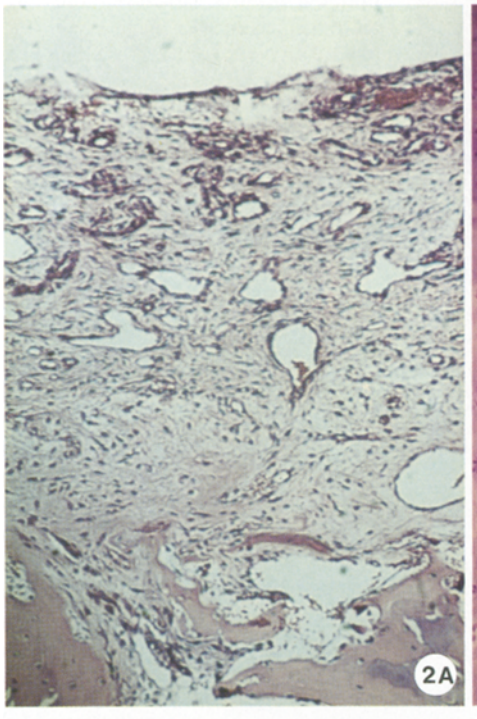
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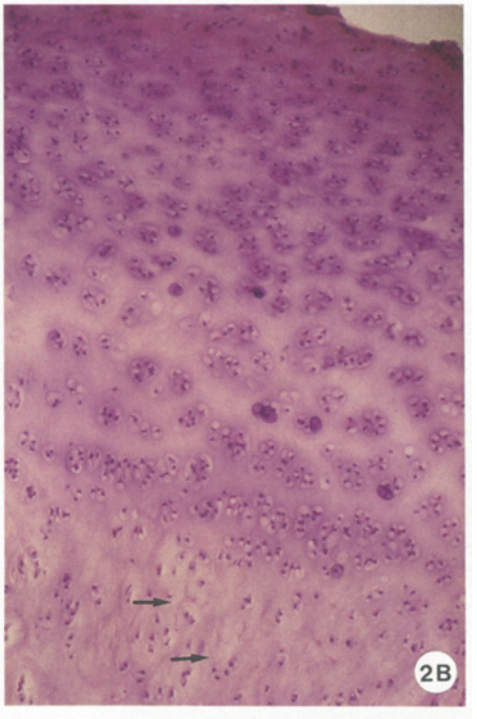
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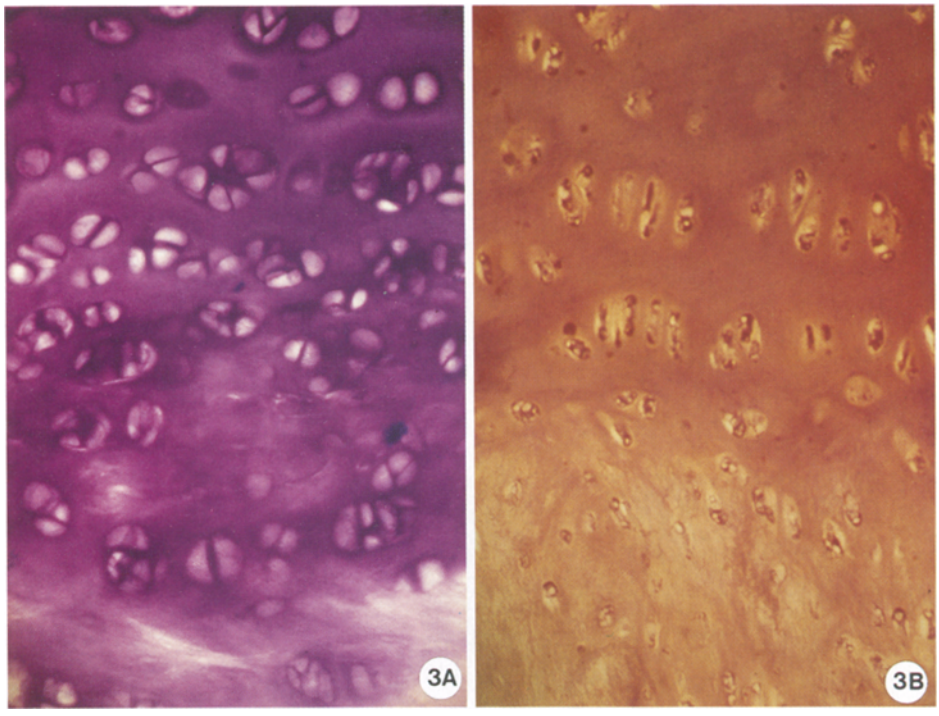

Fig. 3. A A graft from the non-weight-bearing area 4 weeks after transplantation and fixation with fibrin glue. Intense metachromasia in the newly formed tissue is visible. Toluidine blue, ×240.
B Graft from the non-weight-bearing area 8 weeks after transplantation and fixation with collagen sponge. Positive reactivity for matrix glycoproteins and intracellular glycogen is visible. PAS, ×240

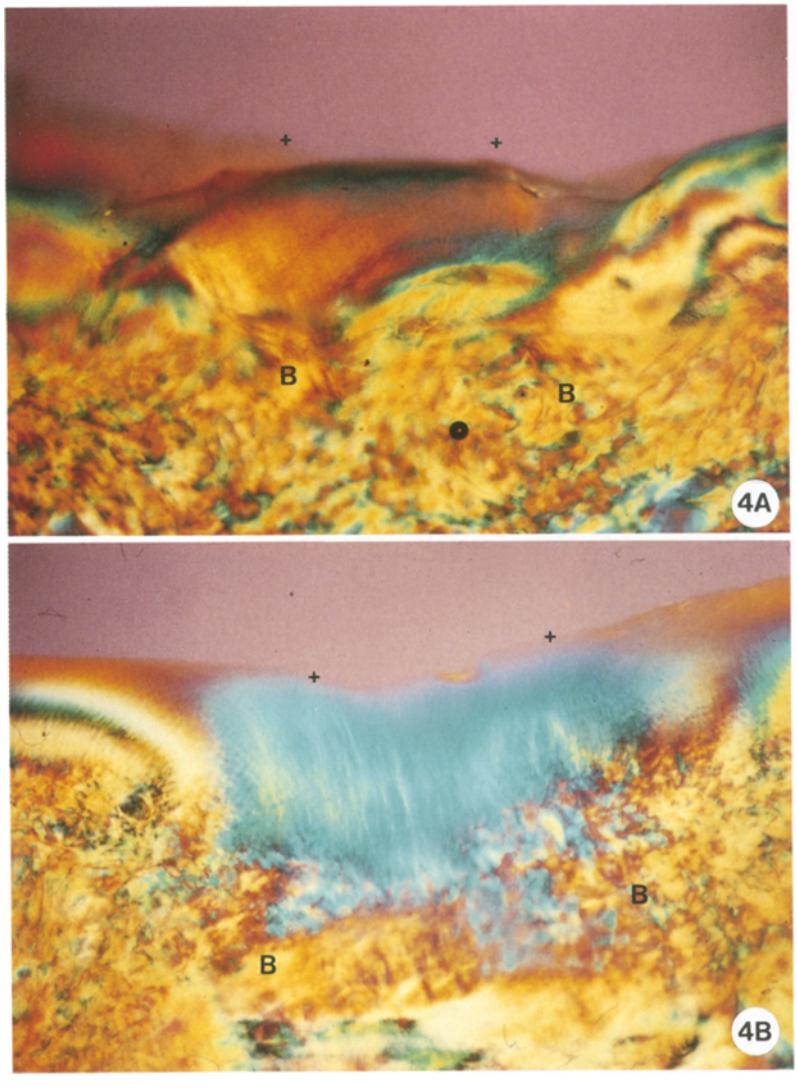

Fig. 4. A Polarized light examination of a control defect (defect only filled with fibrin glue) under polarized light 16 weeks after transplantation into a drill-hole of the non-weight-bearing area. The collagen fibres lack any specific orientation. *B*, Subchondral bone; +, superficial part of the defect. Lens magnification, ×6. **B** Polarized light examination of a transplant from the non-weight-bearing area 16 weeks after transplantation. Collagen fibres appear blue in colour and are arranged in perpendicular orientation. Superficially (+) the fibres appear to attain a horizontal orientation. *B*, Subchondral bone; +, superficial part of the transplant. Lens magnification, ×6

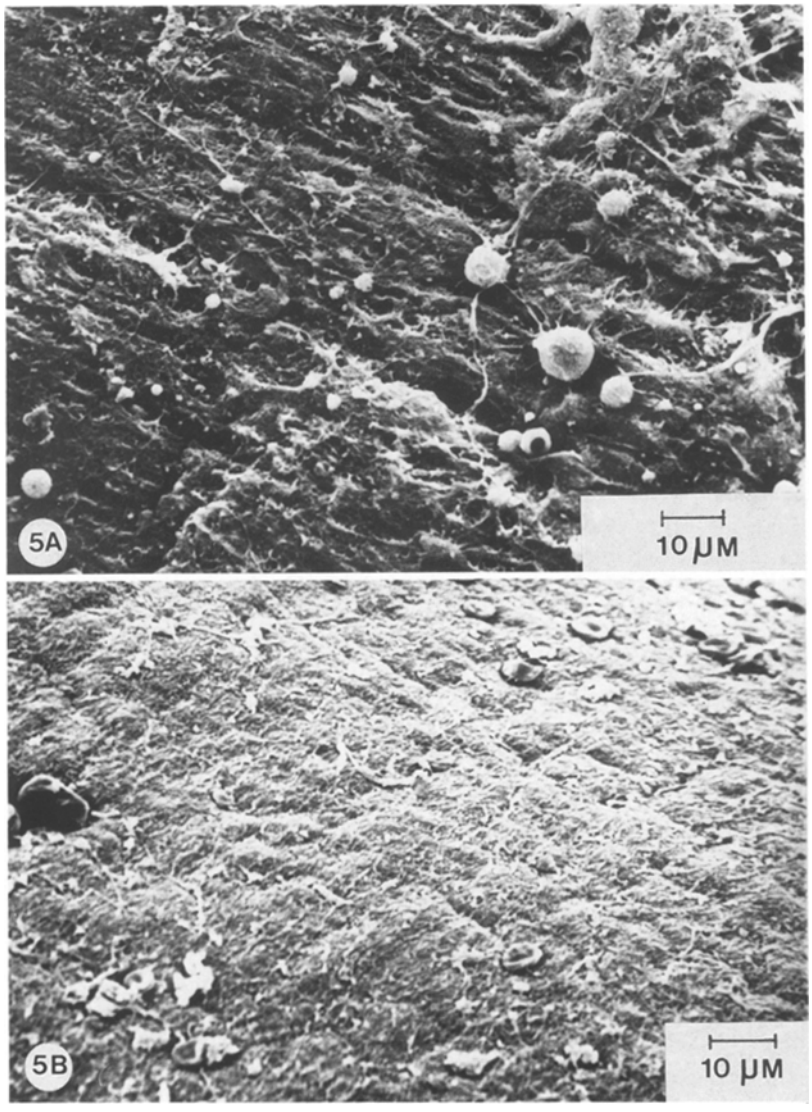

Fig. 5. **A** Scanning electron micrograph of a control defect (without any filling) 8 weeks after drilling. A rough surface with irregular "humps" measuring from 40 µm to 80 µm in diameter along with bigger clefts in between them is visible. **B** Scanning electron micrograph of the articular surface of a graft 16 weeks after transplantation into a defect of the non-weight-bearing area and fixation with fibrin glue. Several "humps" with a diameter of about 20 µm are visible, indicating the existence of underlying chondrocytes. In addition, a regularly structured network of fibres can be seen

which collagen sponges were applied giant cells were observed. The lymphocyte and giant cell aggregation could not be observed after 16 weeks. Twelve-week-old and 16 week-old defects in the non-weight-bearing areas with a hyaline-like cartilage development did not show formation of a tidemark (Fig. 2D).

In contrast, most of the specimens of group A and group B obtained from the weight-bearing sites of the femoral condyle exhibited fibrous cartilage and scar tissue 4–16 weeks after transplantation (Fig. 2C). In only 6 of 48 grafts (3 of group A, 4 of group B) was hyaline-like cartilage formation noticed and all 6 specimens were taken from the anteriorly located drill-hole of the medial condyle. Histological examination of specimens from group C animals (controls) revealed that the defects were filled with scar tissue without any hyaline-like cartilage differentiation in either area (Fig. 2A). The surface of the drill-holes in the non-weight-bearing area was less depressed compared with that in the weight-bearing area. Only 6 of 24 sites in non-weight-bearing areas and in 1 of 24 in the weight-bearing area revealed the development of fibrocartilage.

Histochemically the newly formed cartilage with the histological hyaline-like cartilage appearance reacted positively for acidic toluidine blue (Fig. 3A) and PAS (Fig. 3B), indicative of de novo formation of proteoglycans and glycoproteins. Specimens with a fibrocartilage differentiation were less intensely stained when compared with specimens with a hyaline-like cartilage appearance. Specimens with scar tissue formation did not react with acidic toluidine blue or with PAS.

Under polarized light grafted specimens (groups A and B) of non-weight-bearing areas exhibited similarity to normal non-grafted perichondrium, showing a horizontal fibre orientation in the deeper and more perpendicular orientation in the more superficial part of the transplants, even after 4 or 8 weeks. Twelve weeks after transplantation this horizontal fibre orientation was still visible. Sixteen weeks postoperatively, specimens from the non-weight-bearing area of groups A and B exhibited typical horizontal orientation of collagen fibres in the superficial zone; in the deeper part of the graft perpendicular orientation of the fibres was noted, as is usually seen in normal cartilage (Fig. 4B).

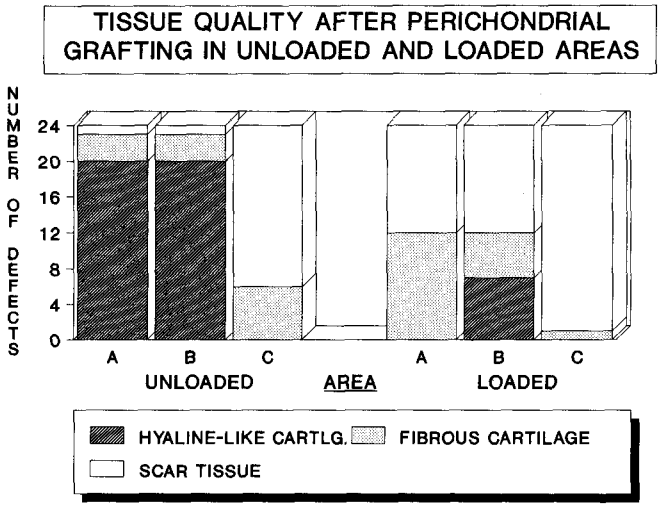

Fig. 6. Histological quality of tissue found in defects after transplantation of perichondrial grafts and in controls (summary of four time intervals). *A*, Fixed with fibrin glue; *B*, fixed with collagen sponge; *C*, control defects

Specimens obtained from weight-bearing areas from groups A and B as well as all specimens from group C revealed a total lack of such organization at all time intervals (Fig. 4A).

On SEM the superficial surface of the articular cartilage in intact sheep knee joints contains "humps" and superficial fissures which are known to be characteristic structural elements of articular cartilage studied in this way (Ghadially and Ghadially 1975; Ghadially et al. 1977; Puhl 1974; Redler 1974). Specimens of the non-weight-bearing area of groups A and B revealed an increasing structural resemblance to that of intact articular cartilage after 4, 8, 12 and 16 weeks (Broom 1982; Bullough and Goodfellow 1968; Speer and Dahner 1979) (Fig. 5B). In specimens of both groups (A and B) taken from the weight-bearing area and in all specimens from group C (Fig. 5A) no regular surface structure was detectable. Specimens from group C showed even deeper fissures and rougher surfaces.

In all specimens (groups A–C) staining for acid and alkaline phosphatase activities were negative in the cartilaginous part. Only at the border of the defects close to the subchondral bone was slight staining for both enzymes noted, indicating the beginning of mineralization at the osteochondral junction (Jeffree 1970). The results are summarized in Fig. 6.

Discussion

Tizzoni (1878) and Doerner (1798) investigated the chondrogenic potential of perichondrium. Other authors have since confirmed the potential of perichondrial grafts to form hyaline-like cartilage in cases of articular cartilage lesions in rabbits and dogs (Amiel et al. 1985a, b; Enkvist and Ohlsen 1979; Engkvist et al. 1979; Kon 1981; Ohlsen and Widenfalk 1983; Skoog et al. 1972, 1975; Woo et al. 1987). Perichondrial grafting has also been successfully performed in humans when the grafts were applied to non-weight-bearing joints such as those in fingers and the wrists (Pastacaldi et al. 1979; Serradge et al. 1984; Sully et al. 1980). Yet, for a clinical application in weight-bearing joints such as knee, ankle and hip, several questions regarding the fixation of the grafts, the time of immobilization as well as the mode of mobilization, [continuous passive motion (CPM) or active motion] have had to be evaluated (Amiel et al. 1985; Engkvist and Ohlsen 1979; Kon 1981; Salter et al. 1975, 1980).

It has been argued that transient immobilization of joints aids the anchoring of transplanted grafts. However, longer periods of immobilization result in the formation of scar tissue (Engkvist and Ohlsen 1979). Early mobilization enhances the growth of the cartilage cells, thus inhibiting the formation of a fibrous scar (Amiel et al. 1985; Salter et al. 1975, 1980) and CPM or active motion has been recommended for the enhancement of hyaline cartilage development (Salter et al. 1975, 1980). The purpose of this experiment was to examine the influence of weight-bearing or weight restriction on the differentiation of perichondrial grafts into hyaline-like cartilage in a large animal model. The main difficulties were related to the postoperative treatment, because animals cannot be mobilized in the same way as humans. Therefore, rigid physiotherapeutic regimens, such as mobilization with CPM, active motion or partial relief from weight-bearing cannot be enforced. In order to simulate a restriction from weight-bearing for the grafts, perichondrial transplantation was performed at the patellar groove which has been reported to never come into contact with another joint surface (Passl et al. 1976). These grafts were compared with those transplanted into the weight-bearing area at the femoral condyle, which is always in contact with the tibial plateau. It was confirmed radiographically that the area defined as "non-weight-bearing" had no contact with any other part of the joint, in flexion or extension and that the area defined as "weight-bearing" was in contact with the tibial plateau.

For application of CPM, which is known to promote the development of hyaline-like cartilage (Amiel et al. 1985a, b, 1988; Salter et al. 1975, 1980) the use of a CPM machine would have been necessary for at least 16 weeks. Because it was difficult to apply a CPM regimen to sheep this technique was abandoned. The sheep were allowed to move freely, with active motion. This meant that the two different articular cartilage areas represent two different conditions of active motion with and without weight-bearing.

Whether the exact conditions of weight-bearing were completely defineable or not, this study clearly demonstrated the differing extent of development of a hyaline-like cartilage which was dependent on the weight-bearing conditions provided in this sheep model. From structural aspects, all micromorphological methods used in this study showed histological and histochemical differentiation from original perichondrial tissue into hyaline-like cartilage up to 16 weeks postoperatively. The development of hyaline-like cartilage followed a typical course. Four and 8 weeks after transplantation remnants of the original fibrous perichondrial part were still visible, whereas after 12 weeks cells of the grafts began to orien-

tate in a more perpendicular fashion in the deeper part and more horizontally in the superficial layer. Grafts also demonstrated an orientation of the fibres similar to the original perichondrial tissue after 4 and 8 weeks, whereas after 12 weeks and even more after 16 weeks fibres began to orientate themselves more like in normal hyaline cartilage with the typical structure of a superficial, intermediate and deep layer. However, the postoperative regimen/included restricted weight-bearing and allowance of motion of the treated joint.

The second purpose of this study was to evaluate two different fixation techniques using either fibrin glue or collagen sponges. Fibrin glue known to be a sufficient resorbable glue which is used clinically for refixation of osteochondrial fractures and/or transplants (Coutts et al. 1984; Gaudernak et al. 1986; Homminga et al. 1989; Kaplonyi et al. 1988; Keller et al. 1986; Passl et al. 1976) although the gluing strength is limited (Claes et al. 1981; Kaplonyi et al. 1988; Keller et al. 1986). Collagen sponges have a good haemostatic function (Schittek et al. 1976). Furthermore, in vitro studies suggest that collagen sponges can promote the differentiation of isolated cartilage precursor cells into cartilage and/or bone cells (Kimura et al. 1984; Maor et al. 1987; Wakitani et al. 1989; Yasui et al. 1982).

In this experiment no cartilage differentiation-promoting effects from the use of collagen sponges were observed when evaluated by cell differentiation, improved fibre orientation of collagen fibres or earlier production of ground substance. In comparison with fibrin glue fixation, the use of collagen sponges appeared to produce a detrimental effect because the quality of the junction between the graft and the subchondral bone and surrounding cartilage was poor; gaps between graft and host were larger and did not show the characteristics of enchondral ossification seen in grafts with glue fixation. The explanation may be that collagen sponges do not fix perichondrial grafts sufficiently well to allow hyaline-like cartilage differentiation, particularly when active motion is applied.

No detectable promoting effect was caused by the collagen, probably because cartilage precursor cells of the perichondrium were already surrounded by the extracellular matrix. This matrix may hinder influences of the collagen sponge to the perichondrium precursor cells. Additionally, the cartilage precursor cells of the perichondrial grafts were already arranged three-dimensionally, which is known to be another promoting effect on cartilage cell differentiation (Wakitani et al. 1989; Yasui et al. 1982).

Clinical investigations such as radiography, MRI or arthroscopy will have to demonstrate whether recommendations for postoperative conditions such as restriction of weight-bearing and/or extent of CPM are transferable from experimental data into clinical practice before weight-bearing is allowed.

The advantages of the use of this technique for the repair of full-thickness articular cartilage defects include the avoidance of immunological complications and the use of tissue which contains intrinsic proliferative potential to improve the ingrowth into cartilage defects with closure of the gap between graft and host. Transplantation procedures using either autologous osteochondrial grafts from the posterior part of the injured knee joint (Yamashita et al. 1991), homologous fresh frozen grafts, or grafts restored otherwise, lack the biological potential to produce hyaline-like cartilage (Hesse et al. 1975; Langer and Gross 1974) and do not promote a junction between grafts and surrounding cartilage. Furthermore, autologous osteochondral grafts taken from the injured joint produce additional defects in the joint which may further promote the development of osteoarthritis (Dürr 1982; Störig 1972; Yamashita et al. 1991) and should not be recommended. Additionally, structural differences of the cartilage layer of these autologous osteochondral grafts regarding thickness and curvature make their application questionable (Störig 1972; Dürr 1982; Yamashita et al. 1991).

Similar problems have to be faced when homologous grafts are used. The limited biological activity and difficulty in choosing grafts with exactly the same geometric characteristics of the recipient area (Hesse et al. 1975) can result in an incongruence of the joint surface which promotes osteoarthritis even after transplantation.

These problems can be avoided if an autologous graft with good regenerative potential such as perichondrium is chosen. Recently, it has been reported that perichondrial grafts in rabbits withstand a postoperative time of 1 year (Amiel et al. 1988), and show similar biomechanical and biochemical variables such as collagen types (Woo et al. 1987; Amiel et al. 1985; Bulstra et al. 1990) when compared with normal hyaline cartilage.

In order to provide optimal healing conditions we would advocate that fibrin glue should be used clinically for perichondrial graft fixation and that weight-bearing is restricted for at least 16–20 weeks.

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