Cartilage resurfacing of the rabbit knee

The use of an allogeneic demineralized bone matrix-autogeneic perichondrium composite implant

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A full-thickness articular-cartilage defect was created in the medial femoral condyles of 32 adult rabbits. The defects were filled with demineralized bone or a composite of demineralized bone and perichondrium. Results of cartilage repair were assessed after 12 weeks of implantation. We conclude that demineralized bone matrix used as a subchondral matrix in a cartilage repair model 1) stimulates and induces subchondral bone ingrowth, 2) provides a surface on which cartilage repair can proceed, and 3) can be utilized as a platform on which perichondrium can be fixed to provide a cellular source for cartilage repair. Repair tissue that developed from perichondrium was thicker, more closely resembled normal articular cartilage, and was of a less fibrous nature than the repair tissue that developed de novo on the demineralized bone matrix.

The quest for techniques to resurface full-thickness articular-cartilage defects has focused on methods that provide genetically acceptable tissues, extrinsic factors for matrix induction, and nutritional factors required for growth of neocartilage (Coutts et al. 1984). One method to achieve these goals has centered on the use of autologous chondrogenic tissues, such as perichondrium (Skoog et al. 1972, Upton et al. 1981, Amiel et al. 1985, 1988, Kwan et al. 1987) and periosteum (O'Driscoll et al. 1988, Salter 1989) for grafting full-thickness defects . Fixation of these materials has involved suturing the tissue directly to adjacent articular cartilage (Coutts et al. 1984) or by fixation to bone plugs as a composite implant (Amiel et al. 1985, 1988). These experiments have been hampered by failures due to detachment of the grafted tissues and resorption of the bone plugs.

Demineralized bone matrix (DBM) has been shown by Urist et al. (1975, 1979) and others (Van de Puhe and Urist 1965, Glowacki et al. 1981a, b, Wittbjer et al. 1982, Reddi 1986) to have substantial osteoinductive activity. Investigations have shown it to be effective when used in surgical reconstructions requiring induction of new bone.

We describe a technique based on the use of an allogeneic DBM-autologous perichondrial composite implant for cartilage resurfacing. DBM was selected as a carrier because it can be easily shaped and contoured to fill a subchondral defect, and it permits secure fixation of perichondrium by simple suturing techniques. The results of repairs using this composite were compared with results of repairs using DBM alone.

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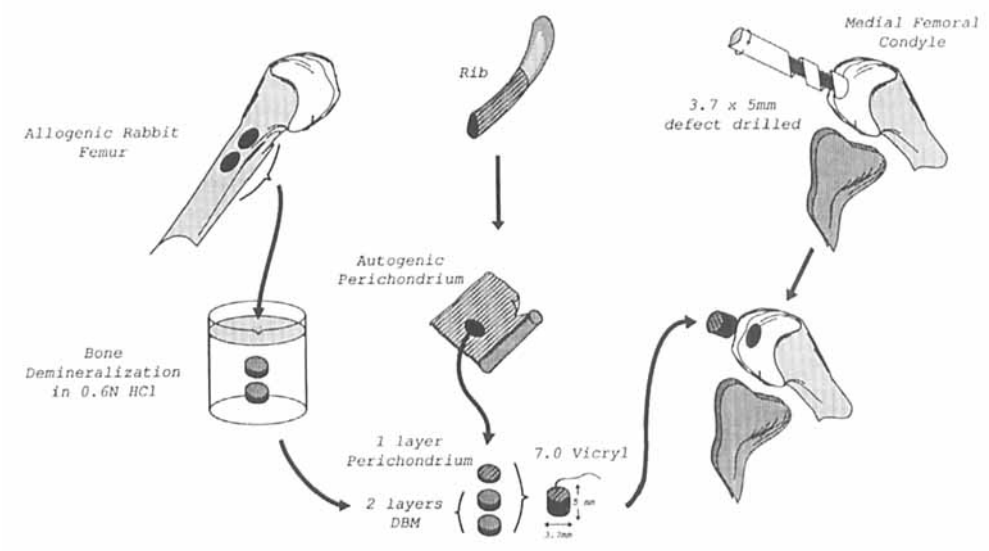

Figure 1. Schematic representation of method employed in the construction and placement of a perichondrium–DBM composite graft into the medial femoral condyle of the rabbit knee. Control knees received DBM alone.

Methods

Skeletally mature New Zealand white rabbits (2,800 ± 400 g) were used. Each animal was anesthetized with ketamine/xylazine (3:1 proportion mixture) injected intramuscularly.

Allogeneic DBM was prepared from 10 rabbits. Both tibias and femurs from each animal were removed under sterile conditions, and all the soft tissues were dissected from them. All the steps of this procedure (Köhler and Kreicbergs 1987) and storage of DBM were done under sterile conditions. Samples were washed free of any remaining soft tissue, bone marrow, and periosteum using deionized water at 4°C for 2 h. The samples were then defatted using a 1:1 mixture of chloroform and methanol at 4°C for 1 h. Demineralization was performed on all the bone with 0.6 N HCl (50 cc/g bone) for 24 h at 4°C with continuous stirring. Samples were then rinsed repeatedly with deionized water (500 cc/g bone) at 4 °C for 1 h until the pH of the wash matched that of water. Representative specimens were studied using an H & E stain with standard histology. All the remaining specimens were stored in sterile glass at –20°C.

The surgical procedure for harvesting perichondrium was performed as follows: The anterior left chest of each rabbit was shaved and prepared with betadine. A longitudinal incision was made along the 10th costal cartilage for a distance of 3 cm. After splitting the overlying musculature, a 2-cm segment of rib cartilage was isolated circumferentially from the surrounding musculature and excised. Care was taken to avoid penetration of the pleural cavity. The excised rib cartilage was pinned to a tongue blade using 25-gauge needles. A longitudinal incision was made along the length of the cartilage using a #15 blade. A periosteal elevator was then used to strip the perichondrium from the cartilage (Figure 1). The perichondrium was kept in sterile saline until ready for implantation.

Immediately after harvest of the perichondrium, the knee joint was opened by a medial parapatellar incision and the patella was dislocated laterally. After maximally flexing the knee, the site for the full-thickness cartilage defect (and subsequent grafting) on the medial femoral condyle was chosen. This was placed as far posterior as possible in the center of this condyle. A 1-mm-diameter drill bit was used to form a starter hole that was widened sequentially using a 2.5-mm standard drill bit, a 3.7-mm standard bit, and finally, a 3.7-mm square-ended drill bit. The resultant defect measured 3.7 mm in diameter and 5.0 mm in depth. The left knee was designated for a DBM and perichondrium composite graft, and the right control knee received a DBM graft alone.

In both groups the DBM was cut using a 3.7-mm internal diameter surgical trephine, producing 3.7-mm discs approximately 2.0 mm in thickness. For the control implant, two of these discs were stacked and skewered with a 25-g needle and then were sutured together in a running fashion using a 7/0 vicryl suture. For the experimental implant, in addition to the two DBM discs, a 3.7-mm-diameter perichondrium disc was placed on top of the DBM with the cambium side facing out away from the DBM. The perichondrial disc was cut manually using the same 3.7-mm trephine. Care was taken during fixation of all the implants to avoid surgical trauma and to place the suture knot beneath the implant surface to avoid intraarticular placement of the knot.

All the animals were returned to cage activity following surgery without immobilization of the operated on limb. Both femoral condyles of 37 animals were operated on. Five animals were excluded (2 infections, 1 self-mutilation, 1 fracture of the condyles at surgery, 1 death), leaving 32 animals to be studied. Three animals were killed at 3 weeks, 3 at 6 weeks, and 2 at 9 weeks to evaluate the progress of bone replacement. Twenty-four animals were killed at 12 weeks for gross evaluation of the repair. Twenty-five knees received DBM alone and 23 knees received DBM and perichondrium. Control and experimental knees of 6 a priori randomly assigned animals of the 12-week group were studied histologically. All of the 3-, 6-, and 9-week animals were evaluated histologically. The remainder of the knees were frozen for future alternative testing. Evaluation was performed by gross observation and histology using H & E and safranin-O staining.

Gross appearance. At the time of postmortem examination, the appearance of the implant (graft) site was evaluated grossly. A determination of "biological acceptability" was made. Defects that were filled with neocartilage that made contact with the periphery and that had an articular surface contiguous with the surrounding articular cartilage were considered "biologically acceptable" (Amiel et al. 1985). This identified specimens that represented technically adequate results. This was not meant to imply perfect reconstruction of articular tissue, but rather to eliminate specimens that were failures of the surgical technique.

Histologic evaluation was done on sagittal sections of the implant region on the condyle. Both the H & E- and safranin-O-uptake stained sections were reviewed and graded using a system designed to measure tissue attachment and confluence (attachment) and the quality of the neocartilage at each repair site (morphology). Attachment was scored for confluence with the surrounding cartilage (1 point), basilar attachment of the neocartilage (1 point) and peripheral attachment of the neocartilage (1 point for each of 2 sides of the sagittal section) for a total of 4 possible points. Morphology was grade for the quality of the neocartilage by safranin-O uptake (1 point), cellular arrangement (1 point), height of the neocartilage column (1 point), and orientation of the neocartilage cells (1 point) for a total of 4 possible points and a combined total of 8 possible points (Kwan et al. 1987).

In evaluating DBM incorporation into bone, a histologic grading system was utilized: acellular with no new bone formation (0 points), a few viable cells with less than 30 percent replacement of the DBM (1 point), peripheral incorporation with revascularization, increased numbers of viable cells, and 30–60 percent replacement of the DBM (2 points), and trabecular organization with revascularization with greater than 60 percent replacement of the DBM (3 points).

Results

Repairs with implantation of DBM alone had biological acceptability in 21/25 condyles and repairs with DBM and perichondrium had 20/23 acceptance rate. The DBM alone showed neocartilage repair tissue ranging in color from pink to white, as well as surface patterns ranging from smooth to convoluted. Confluence with the surrounding cartilage was generally completed. The DBM with perichondrium showed somewhat the same range of gross findings, but tended toward having a thicker repair tissue (Figure 2). Perichondrium produced a more convoluted surface appearance.

There were a total of 7/48 biologically unacceptable knees. Four cases occurred with implantation of DBM alone and were due to lack of repair tissue proliferation and poor incorporation of the DBM. Three cases occurred with DBM and perichondrium due to complete failure of graft attachment and proliferation, and were associated with poor DBM incorporation. In 1 of these cases, failure was also related to medial placement of the defect, such that the cruciate ligaments interfered with graft proliferation. In another case, lack of incorporation was thought to be due to a low-grade infection.

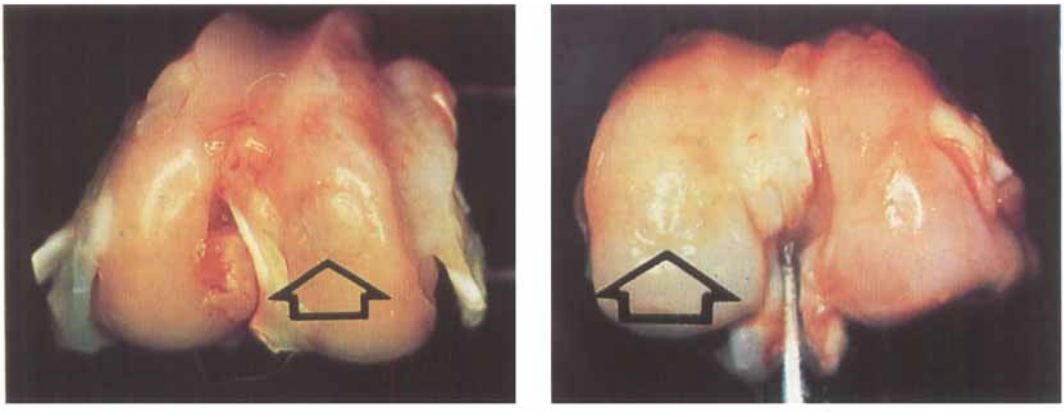

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Figure 2. Gross specimens 12 weeks postsurgically.

A. Result with DBM alone.

B. Result with perichondrium–DBM composite. Confluence repair was evident in both sides (arrows).

Histology

Repair with the DBM alone scored a mean total of 3.8 (1.5–6.5) points for both attachment and morphology (Table 1), representing a neocartilage repair close to that of mature cartilage without any chondrocyte-cell source having been implanted with the composite graft. These specimens had some areas of well-differentiated neocartilage but other areas where a more fibrous neocartilage was noted (Figure 3). Repairs with DBM and perichondrium had a mean total score of 4.2 (2–8) points for attachment and morphology, and demonstrated a repair tissue

Table 1. Histologic scoring results (N 4)

Criteria	Maximum	DBM alone*	DBM and perichondrium
Repair tissue attachment	4	2.3 (0–4)	2.2 (1–3.5)
Morphology	4	1.5 (0–3)	2.1 (1–4)
DBM incorporation	3	2.1 (0.5–3)	2.2 (1–3)

* Median (range).

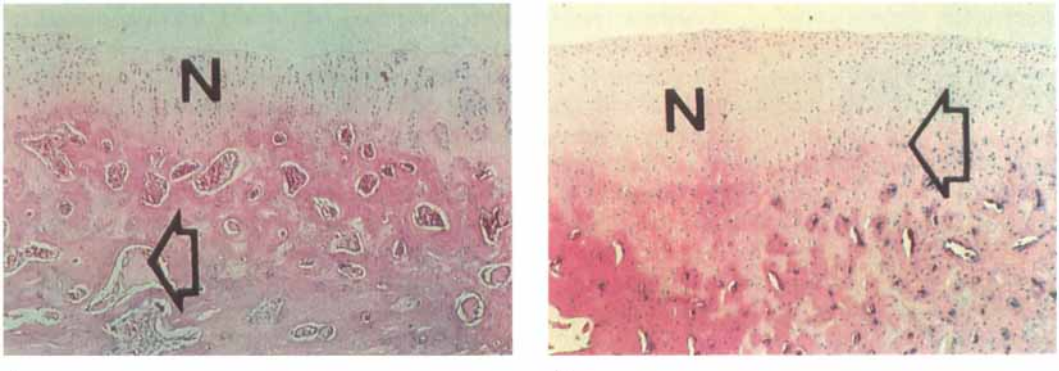

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Figure 3. Histologic specimens 12 weeks postsurgically.

A. Best result of de novo neocartilage (N), which developed over DBM alone. Residual DBM is still evident(clear arrow).

B. Neocartilage (N), which developed from DBM-perichondrium composite. Neocartilage appears morphologically similar, although thicker than normal cartilage. The tidemark is reestablished (clear arrow).

that had differentiated cells and an organization and orientation that more closely approached that of adjacent normal cartilage tissue.

Comparing the two groups, histologic scores for attachment were equivalent, with greater variability seen in the repair tissue that developed de novo and that which developed from the perichondrium. The morphology scores were higher for the repair tissue that developed from perichondrial grafts (Table 1). Perichondrial repair tissue was also thicker and of a less fibrous nature than the de novo repair tissue, which was less differentiated. Both groups demonstrated variable scores of bone incorporation, but scores were similar between the two groups. Overall, there was evidence of a gradual replacement of DBM by bone over the 12-week period. The tidemark became better delineated as the neocartilage and subchondral bone differentiated. Repairs of poorer quality showed less complete DBM replacement as well as a more fibrous appearance of the cartilage repair by 12 weeks. Specimens from both groups often showed total replacement of the DBM in the subchondral region by new bone accompanied by the formation of a smooth and well-demarcated tidemark between the subchondral bone and the neocartilage.

Discussion

Grafting of perichondrium to full thickness articular-cartilage defects has produced a mature, well-differentiated neocartilage in these defects (Engkvist and Wilander 1979, Ohlsen 1976, Skoog et al. 1975, Haas 1914, Kon 1981). However, the failure rates of these procedures are high due primarily to detachment of the grafted tissues (Kon 1981, Coutts et al. 1984, Amiel et al. 1985, Kwan et al. 1987 Amiel et al. 1988). Direct fixation by suturing to adjacent normal cartilage is not clinically applicable due to injury to healthy tissue and technical difficulties. Efforts to use autogenous bone plugs as a graft carrier have been troubled by inadequate fixation of perichondrium to this bone. Host replacement of the bone plug and its incorporation have been slow, thereby denying the perichondrium a viable surface on which to attach.

DBM is a firm material that can be contoured easily, and perichondrium can be attached to DBM using a small suture needle, thus facilitating better fixation of the graft. Therefore, DBM can provide a good platform for carrying chondrogenic tissue for implantation.

In this study, we evaluated cartilage repair at 12 weeks after implantation. A biological acceptance rate of 0.9 for DBM and perichondrium and of 0.8 for DBM alone shows significant improvement over our previous work (Amiel et al. 1985, Kwan et al. 1987, Amiel et al. 1988) using this same model, but using autogenous bone plugs instead of DBM, which had a biological acceptance rate of 0.6. This improvement may reflect the effect of the stronger osteoinductive properties of DBM and suggests possible chondrogenic properties of DBM compared with bone alone.

The neocartilage overlying the DBM implants was less differentiated than that over the perichondrium-autogenous bone composite grafts and more closely resembled fibrocartilage. DBM may provide a platform for marrow cells or adjacent healthy cartilage to migrate and also may contain factors contributing to chondrogenic induction. The presence of a chondrogenic cell source (perichondrium) contributed to a higher quality neocartilage repair, albeit with a variable attachment to the bone support.

The subchondral bone incorporation of the DBM progressed rapidly with almost complete replacement by 12 weeks. Histologic evidence of a smooth distinct tidemark between the new subchondral bone and well-differentiated neocartilage provides evidence of the osteogenic and chondrogenic potential of these composite grafts.

We believe this technique, with adequate development, could provide a method for clinical articular-cartilage resurfacing.

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