REPAIR OF CARTILAGE LESIONS USING BIOLOGICAL IMPLANTS

A COMPARATIVE HISTOLOGICAL AND BIOMECHANICAL STUDY IN GOATS

B. F. SHAHGALDI, A. A. AMIS, F. W. HEATLEY, J. McDOWELL, G. BENTLEY

From Imperial College, St. Thomas' Hospital and the Institute of Orthopaedics, London

We report the experimental use of three different biological implants to restore articular surface defects: glutaraldehyde-fixed bovine meniscal xenograft, glutaraldehyde-fixed bovine costal cartilage xenograft, and viable osteochondral allografts. The grafts were implanted in the knees of 19 goats who were allowed free-field activity and were studied for up to one year.

The natural articular surfaces of meniscal fibrocartilage provided excellent articular surfaces at all times. Equally good articular surfaces were restored by host tissue growth covering costal cartilage grafts at six months, but by 12 months this surface had degenerated. The majority of the allografts survived and integrated with the host at six months, but many showed signs of failure at 12 months. Only three out of seven ungrafted defects healed completely at six months and the healed surfaces were degenerating at 12 months.

Articular cartilage has limited capacity for repair and once damaged it does not heal to recover the lost surface (Mankin 1982). However, if the subchondral bone is also damaged, repair tissue which has been reported variously as fibrous, fibrocartilaginous, or of hyaline cartilage will develop from the subchondral bone (DePalma, McKeever and Subin 1966; Salter et al 1980). This tissue is biochemically and biomechanically different from normal hyaline articular cartilage (Coletti, Akeson and Wood 1972).

In view of these findings it is desirable to find satisfactory ways of repairing articular surfaces in conditions such as chondromalacia, osteochondritis dissecans, osteochondral fractures and possibly early osteoarthritis. In recent years, several studies have reported the use of natural (viable and nonviable) and synthetic materials in both animal and human trials. The materials used include viable osteochondral allografts

(Gross et al 1976; Aston and Bentley 1986), glutaraldehyde-fixed bovine meniscus (Heatley and Revell 1985), glutaraldehyde-fixed collagen sponge (Speer et al 1979), and carbon fibre sponge (Minns, Muckle and Donkin 1982). However, variations in experimental protocol have meant that the results of using different materials cannot be compared. Furthermore, no attempt has been made to define or control the biomechanical environment of the implants, although this is a major influence on tissue responses (Shahgaldi, Amis and Heatley 1989).

We report a comparative study of three different grafts: glutaraldehyde-fixed bovine meniscal xenograft, glutaraldehyde-fixed bovine costal cartilage xenograft and culture-medium-preserved, viable osteochondral allografts. In the first two the technique of tanning with glutaraldehyde has the effect of producing cross-links within the collagen, toughening its texture and also diminishing its antigenicity.

Our aims were to examine the influence on biological repair of the mechanical fit of the graft, and to assess the effectiveness of the three different grafts in comparison with ungrafted defects.

MATERIALS AND METHODS

Glutaraldehyde-fixed xenografts

Preparation. Meniscal xenografts were prepared from bovine menisci, obtained from mature animals two to three days post-mortem, and placed in a 1% aqueous solution of glutaraldehyde, neutralised to pH 7.4 with 0.2 M phosphate buffer. After 24 hours, 6 mm diameter plugs, 6 mm in depth, were removed from the partially-

B. F. Shahgaldi, MSc, PhD, Research Assistant
A. A. Amis, PhD, CEng, MIMechE, Senior Lecturer
Biomechanics Section, Department of Mechanical Engineering,
Imperial College of Science, Technology and Medicine, Exhibition
Road, London SW7 2BX, England.

F. W. Heatley, FRCS, Reader in Orthopaedic Surgery
St. Thomas' Hospital, London SE1 7EH, England.

J. McDowell, BSc, Research Student
G. Bentley, ChM, FRCS, Professor of Orthopaedic Surgery
Institute of Orthopaedics, Royal National Orthopaedic Hospital,
Brockley Hill, Stanmore, Middlesex HA7 4LP, England.

Correspondence should be sent to Dr B. F. Shahgaldi.

© 1991 British Editorial Society of Bone and Joint Surgery
0301-620X/91/1036 \$2.00
J Bone Joint Surg [Br] 1991; 73-B:57-64.

fixed tissue. One of the articular surfaces of the meniscus was preserved on each plug to form the articular surface of the graft. The plugs were placed in 1% neutral glutaraldehyde solution for one week to ensure complete fixation, then rinsed thoroughly in distilled water, using a vacuum to extract unreacted glutaraldehyde before rehydration. Rinsing and extraction were repeated until there were no traces of glutaraldehyde in solution: the extracted fluid was periodically tested for the presence of glutaraldehyde using 2,4-dinitrophenylhydrazine (Shahgaldi 1988). The plugs were stored in full strength Ringer's solution and sterilised using 2.5 Mrads of gamma irradiation. Bovine costal cartilage xenografts were prepared by the same method.

placed about 0.25 mm below the level of the articular cartilage surface.

Viable allografts

The osteochondral allografts were plugs of 5 mm diameter and 4 to 6 mm in length. They were removed from the load-bearing areas of the patellae, patellar grooves and femoral condyles of mature goats under general anaesthesia (the donors were recipients of the xenografts). These plugs were placed in universal bottles containing Hams F12 (Nutrient Mixture) medium supplemented with 10% fetal calf serum, 1% antibiotic/antimycotic, 1.4 mM L-glutamine, 150 µg/ml of ascorbic acid and 1% Hepes buffer (all Gibco-Europe). They were stored at 4°C for 14 days before implantation into goats.

Fig. 1a

Fig. 1b

Figure 1a – An example of the articular surfaces obtained with a meniscal xenograft, showing a successful patellar graft at 12 months. The dark colour of the graft is due to glutaraldehyde staining. Figure 1b – The articular surface provided by the healing of an ungrafted defect at six months.

Biomechanical fit. A laboratory study was designed to see whether the two different xenografts could be matched precisely for indentation hardness and contact pressure with the articular cartilage of cadaveric knees from goats. To model the repair of a lesion, a circular hole was cut into the joint surface and a core of cartilage and bone was removed. The xenografts were used to plug such holes in load-bearing areas in the patellae, patellar grooves and the femoral condyles. The tightness of diametrical fit and prominence in relation to the joint surface were varied in an attempt to obtain the same indentation hardness and contact pressure as the surrounding articular cartilage (Shahgaldi et al 1989).

It was found that meniscal xenografts inserted into osteochondral defects with 0.3 mm diametral interference and approximately 0.25 mm proud of the cartilage surface had similar characteristics to the surrounding articulation (optimised position). The costal cartilage xenografts were harder than goat articular cartilage so had similar contact pressure to the surroundings when

Cellular viability was assessed, using plugs which were obtained as above, after one, seven and 14 days storage by incubating them in 1 ml of the same medium containing 20 µCi $^{35}\text{S}\text{O}_4$ (to indicate matrix production of proteoglycan) at 37°C for three hours. After labelling, the plugs were washed four times in 10 ml (10 ml per wash) of sterile phosphate buffered saline (PBS) for 24 hours on a roller to remove unbound label. Individual plugs were then digested in papain using the method of Royce and Lowther (1979). After digestion the volumes were equilibrated to 1 ml; 100 µl was added to 4 ml Aquasol-2 (New England Nuclear) and radioactivity counted in a Minaxi Tricarb 4000 (Packard) Counter. The remaining digestion fluid was stored frozen for DNA analysis. Measurement of total DNA/skeletal rudiment was carried out using the ethidium bromide method of Royce and Lowther (1979).

Surgical procedure

The grafts were used in the left knees of 19 mature goats, the right knees being kept intact as controls. Each of 15

goats received three grafts and one ungrafted defect (six goats had 18 costal cartilage xenografts, six goats had 17 viable allografts, three goats had nine meniscal xenografts). Additionally, four goats received 16 meniscal xenografts, four in each goat.

Only one type of graft was used in each goat. They were inserted into surgically created osteochondral defects in the medial facet of the patella, the lateral facet of the patellar groove, and the anterior end of the medial femoral condyle, with the lateral femoral condyle containing an ungrafted defect (Figs 1 and 2). The defects were standardised so that the diameter and the depth were constant.

Fig. 2

A typical example of the articular surface obtained at six months with a viable allograft in the medial femoral condyle (left) compared with a partially healed ungrafted defect in the lateral femoral condyle (right). The graft diameter was 5 mm.

To examine the effect of mechanical optimisation on biological repair the *meniscal xenografts* were inserted into defects which were 0.3 mm undersized in diameter and at three different levels with respect to the surrounding articular surface: sunken, level with, or in the optimised position of 0.25 mm above the surface, as had been determined by laboratory tests. The *costal cartilage xenografts* were implanted in the optimised positions only, tight-fitting and 0.25 mm below the surface.

The *viable allografts* were inserted into tight-fitting defects with their articular surfaces level with the surroundings. These grafts were morphologically matched with the implant sites, for example, a plug that was removed from a patella was implanted in another patella in the same site so that the articular cartilage thickness and the subchondral bone were matched.

Limbs were not immobilised postoperatively and the animals were allowed free-field activities as soon as they were walking normally, two to four weeks after operation.

Laboratory evaluation

At the end of each trial the animals were killed and the knees were examined for wear and photographed. The articular surfaces of the operated and control knees were tested for indentation hardness and contact pressure and processed for histology.

RESULTS

Because of the large size of the grafts it was often possible to tell from the macroscopic appearances whether they had been successful, but they were assessed macroscopically and microscopically using the following criteria:

Success. There was a congruent surface continuous with the surrounding articular surface, restored either by the graft or by host tissue overgrowth. The graft was intact and had bonded firmly to the host by either bony junction or fibrous tissue growth or both. Its indentation hardness and the contact pressure of the joint were similar to those of the grafts implanted in the control knees and tested in the laboratory. In the case of ungrafted defects a repair was successful if the defect had completely healed with cancellous bone, a subchondral bone plate and articular surface coverage. The host articular cartilage was normal.

Failure. A graft was considered to be unsuccessful if any one of the failure criteria shown in Table I occurred.

Macroscopic appearance. Typical examples of the articular surfaces obtained from different resurfacing methods are shown in Figures 1 and 2. At six months, all six meniscal xenografts set at optimised positions at operation had subsided to form congruent surfaces. Two of the seven set level with the surrounding surface had maintained their height and five had sunk. The sunken xenografts were covered by a layer of host tissue forming macroscopically depressed healed surfaces.

At 12 months, five of the six xenografts set in the optimised position had subsided and formed congruent surfaces. One sank to just below the cartilage level.

Costal cartilage xenografts had restored the articular surface with the implant surface in 10 cases and by host tissue overgrowth in eight. The host tissue overgrowth formed intact articular surfaces continuous with the surroundings at six months but there was a tendency for this layer to degenerate by 12 months, the changes being more pronounced in the tibiofemoral than in the patellofemoral articulations.

The successful allografts (Table I) had remained in the congruent positions set at operation; their articular surfaces were indistinguishable from the surrounding host tissue at all periods (Fig. 2). The articular surfaces of the failed allografts had fibrillated and worn (Table I).

The areas of the ungrafted defects were grossly discernable at all periods of review. Only three of seven defects had completely healed at six months, with complete but irregular articular surfaces (Fig. 1b). The surfaces of the unsuccessful specimens had only partially healed and macroscopically there was an obvious defect

(Fig. 2). Three of eight defects had completely healed at 12 months with features similar to those described for successful six-month specimens.

Histology. At six months the host-graft junctions of the meniscal xenografts with congruent surfaces appeared as bone or a mixture of bone and dense fibrous tissue firmly uniting the graft to the host (Fig. 3). The amount of bone

at the junctions increased with time so that those of the 12-month specimens were mainly bone. The sunken grafts were lying in cavities surrounded by loose fibrous tissue with poor attachment to the grafts (Fig. 4). In general, the grafts remained intact and inert, chemically and biologically.

The host tissue covering the costal cartilage xeno-

Fig. 3

Fig. 4

Figure 3 – a meniscal xenograft in the femoral condyle, a typical example of an optimally positioned graft at 12 months. The gap on the right was caused by shrinkage during specimen preparation. Figure 4 – xenograft in a sunken position is surrounded by loose fibrous tissue growth at six months (Masson's trichrome).

Table I. Results of different resurfacing methods

Graft	Follow-up (months)	Successful (number)	Host response	Comments
Meniscal xenografts raised* level* below*	6	6/6	Normal	Failure occurred in sunken grafts
		4/7		
		2/6		
Meniscal xenografts (Optimal position)	12	5/6	Normal	Failure occurred in sunken grafts
Costal cartilage xenografts (Optimal position)	6	8/9	Normal	The failed implant had fractured horizontally 2 mm below the surface
	12	6/9	Normal	Failures due to fibrillation of cartilage and breakdown of host tissue growth
Osteochondral allografts (Morphologically matched)	6	5/9	Normal in 8/9	Failures due to graft bone resorption (2), bone survival but cartilage degeneration (2)
	12	2/8	Normal in 5/8	Failures due to graft bone resorption (2) graft cartilage degeneration (4)
Ungrafted defects	6	3/7	Normal in 6/7	Failures due to incomplete filling of the defects
	12	3/8	Normal in 4/8	Failures due to incomplete filling of the defects, fibrillation and breakdown of the repaired articular surface

* in relation to the host articular surface

Fig. 5

Degenerated host tissue growth covering a costal cartilage xenograft at 12 months (Masson's trichrome).

Fig. 6

A successful allograft at 12 months: the graft cartilage is similar to the host cartilage (Masson's trichrome).

Fig. 7

A successfully healed ungrafted defect at six months (Masson's trichrome).

Fig. 8

A healed defect with degenerated articular surface at 12 months (Masson's trichrome).

grafts was cartilage-like at the edges but fibrocartilaginous at the centre. At six months this layer was intact, but at 12 months it had degenerated (Fig. 5). At six months the host-graft junctions were made up of bone or a mixture of bone and fibrous tissue firmly uniting the host to the graft. The consistency and thickness of the fibrous tissue varied within a specimen and between specimens, but it was always less than 2 mm thick. At 12 months the junction consisted mainly of bone, and in general the grafts had remained intact.

The articular cartilage and subchondral bone of the successful allografts were similar to those of the adjacent normal host at all times, although the host-graft cartilage junction had not healed, and had been opened up by the histological processing (Fig. 6). In failed cases either the articular surfaces had become fibrillated and worn, while

the subchondral bone survived and integrated with the host, or else the bone was dead.

The articular surfaces of the successfully healed, ungrafted defects (Fig. 7) were made up of intact fibrous tissue. The subchondral bone plates were deranged and varied in thickness within a specimen and between specimens (thinner or thicker than that of the adjacent host). The failed defects were only partially filled, often with a mixture of dense and loose fibrous tissue. The number of successful cases did not improve significantly with time (Table I). Furthermore, at 12 months the repaired articular surfaces were breaking down (Fig. 8).

Mechanical tests. If the xenografts remained at their optimised levels, they transmitted load, but they became nonload-bearing if they sank. The indentation hardnesses (Fig. 9) and the contact pressures of the successful

allografts and their surrounding cartilage (Fig. 10) were similar to those of the unoperated control knees at six months. Those of the 12-month specimens could not be determined because too few specimens were successful.

The contact pressures of the ungrafted defects were not normal at any time. They varied within a specimen and between specimens; there were high and low pressure regions on the surfaces of individual defects (Fig. 10).

cancellous bone. Our findings are in agreement with those of Convery, Akeson and Keown (1972) who found that, in horses, the repair of osteochondral defects in the distal femur was much better in small defects than in larger ones.

Although the number of successful allografts decreased considerably with time, those that remained successful at 12 months produced articular surfaces that

Fig. 9

Indentation hardness of grafts related to time from implantation and compared with normal cartilage surfaces. Indentation is depth of penetration of a 3.2 mm spherical indenter with an applied load of 9.2 N after one second.

Host response. The host tissue surrounding and opposing all graft types remained normal in all of the joints except where it was covering the sunken allografts and around some of the ungrafted defects at 12 months. In these areas, the tissues had become fibrous, contained clusters of chondrocytes, and had fibrillated and cracked.

DISCUSSION

Our study has shown that ungrafted osteochondral defects, 6 mm in diameter and in depth, often failed to heal. In addition, the fibrous reparative tissue at the surface underwent degeneration between six and 12 months. This late failure of the repair tissue could have been caused by absence of mechanical support. Contact pressure mapping showed that the surrounding host cartilage softened with time, and also that the reparative articular surfaces were either harder or softer than the normal articular surfaces at the equivalent places in unoperated knees (Figs 9 and 10). This was related to variation in thickness of the reparative tissue at the surfaces and either an abnormally thick or an absent subchondral bone plate with voids in the underlying

were macroscopically and microscopically indistinguishable from the host tissues. The articular cartilage remained separate from the surrounding host cartilage, but the underlying bone had completely united with that of the host, no host-graft junction could be seen.

We made every effort to match the allografts biomechanically with the host tissue by selecting animals of the same breed, age, weight and sex and by site-matching and positioning the grafts with their articular surfaces level with their adjacent host tissue. Since the thickness of goat articular cartilage, like that of man, varies considerably in different anatomical sites, we used site-matching to ensure that each graft had similar cartilage thickness and followed a similar curvature to the surroundings. Since articular cartilage takes its nutrition mainly from synovial fluid under pressure (Greenwald and Haynes 1969; Mow and Torzilli 1975), it is possible that the articular cartilage of sunken grafts was unable to achieve adequate nutrition and reverted to fibrous tissue.

The xenografts, when positioned in the optimal positions, were invariably successful (Table I). The softer meniscal xenografts, positioned slightly proud of the host

articular surface, subsided to produce congruent surfaces continuous with the surroundings. A very small change in the level (around 2 mm) caused a profound change in tissue reaction (see Figs 3 and 4).

The harder costal cartilage xenografts, set at a lower level, often became covered by host tissue overgrowth; this produced an excellent articular surface at six months but degenerated by 12 months. Both xenograft types became firmly embedded, surrounded by host bone and a small amount of fibrous tissue. They remained intact as space occupiers but were impervious to the host; there was no penetration of host tissue into the grafts other than within superficial crevices in their surfaces. No damage to the host, such as articular wear or bone resorption, arose from adverse chemical, biological or mechanical reaction to the xenografts.

of these grafts, a factor not studied previously. Although some of the osteochondral allografts gave excellent results, there was a worrying proportion of failures. This may be linked with the loss of cellular viability caused by two weeks storage in culture medium (Table II). A further factor is that cartilage did not heal across its interface, despite the close fits we attained. Aston and Bentley (1986), have reported similar findings; the split which remains around the graft cartilage could act as a focus for later degeneration.

The meniscal xenografts gave reliable results, though one possible problem is that glutaraldehyde-fixed xenografts may eventually degrade in a way which does not allow maintenance of an intact articulation. Trials lasting several years or more must follow, but one-year results show that this implant is tough, durable and bonds firmly with the host.

Fig. 10

Contact pressure results, showing pressure maps obtained by using Prescale Pressure Detection Film (Fuji Film Co). The contact pressure is shown by the colour density of the contact areas.

Glutaraldehyde-fixed xenografts have been reported to cause cytotoxic and synovitic reactions in joints (Van Steensel et al 1987), but the method they used meant that there was free unreacted glutaraldehyde at implantation. Our use of a vacuum extraction and rehydration regime avoided such problems.

Our work has shown clearly that both the tissue reaction and the pressure distribution across the articulation depend critically on the accuracy of the placement

Table II. Results of cellular viability assays

	Days stored		
	1	7	14
Chondrocyte count (cpm/100 µl of digest)	13 571	669	256
DNA content (µg/ml)	23	31	20

Conclusions. We concluded that:

- 1) large osteochondral defects do not heal;
- 2) an optimum mechanical position of a xenograft enhances biological repair;
- 3) costal cartilage xenografts have no future, there is late breakdown of their articular tissue covering;
- 4) meniscal xenografts, when implanted in ideal conditions, regularly produced excellent results, and were easy to prepare and store;
- 5) viable allografts produced excellent results in some cases, but we were concerned at the proportion of failures and feel that further work on storage methods is needed.

We are grateful to the Arthritis and Rheumatism Council of Great Britain for generously funding this project. We also thank Dr C. Archer, Dr J. Pringle, Miss M. Brown and Mr G. Batchelor of the Royal National Orthopaedic Hospital, Stanmore and Dr P. Clay and Mr D. Green and his assistants of Imperial College for their help with this project.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

REFERENCES

- Aston JE, Bentley G. Repair of articular surfaces by allografts of articular and growth-plate cartilage. *J Bone Joint Surg [Br]* 1986; 68-B:29-35.
- Coletti JM, Akeson WH, Wood SL-Y. A comparison of the physical behavior of normal articular cartilage and the arthroplasty surface. *J Bone Joint Surg [Am]* 1972; 54-A:147-60.

- Convery FR, Akeson WH, Keown GH.** The repair of large osteochondral defects: an experimental study in horses. *Clin Orthop* 1972;82: 253-62.
- DePalma AF, McKeever CD, Subin DK.** Process of repair of articular cartilage demonstrated by histology and autoradiography with tritiated thymidine. *Clin Orthop* 1966; 48:229-42.
- Greenwald AS, Haynes DW.** A pathway for nutrients from the medullary cavity to the articular cartilage of the human femoral head. *J Bone Joint Surg [Br]* 1969; 51-B:747-53.
- Gross AE, Langer F, Houpt J, Pritzker K, Friedlaender G.** Allotransplantation of partial joints in the treatment of osteoarthritis of the knee. *Transplant proc* 1976; 8(2 Suppl 1):129-32.
- Heatley FW, Revell WJ.** The use of meniscal fibrocartilage as a surface arthroplasty to effect the repair of osteochondral defects: an experimental study. *Biomaterials* 1985; 6:161-8.
- Mankin HJ.** Current concepts review. The response of articular cartilage to mechanical injury. *J Bone Joint Surg [Am]* 1982; 64-A:460-6.
- Minns RJ, Muckle DS, Donkin JE.** The repair of osteochondral defects in osteoarthritic rabbit knees by the use of carbon fibre. *Biomaterials* 1982; 3:81-6.
- Mow VC, Torzilli PA.** Fundamental fluid transport mechanisms through articular cartilage. *Ann Rheum Dis* 1975; 34(suppl):82-93.
- Royce PM, Lowther DA.** Fluorimetric determination of DNA in papain digests of cartilage, using ethidium bromide. *Connect Tissue Res* 1979; 6:215-21.
- Salter RB, Simmonds DF, Malcolm BW, Rumble EJ, MacMichael D, Clements ND.** 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-A:1232-51.
- Shahgaldi BF.** Biomechanical optimisation of an osteochondral replacement biomaterial. PhD Thesis 1988, London University.
- Shahgaldi BF, Amis AA, Heatley FW.** Mechanical behaviour of an osteoarticular replacement material. In: Yettram AL, ed. *Material properties and stress analysis in biomechanics*. Manchester University Press, 1989:139-49.
- Speer DP, Chvapil M, Volz RG, Holmes MD.** Enhancement of healing in osteochondral defects by collagen sponge implants. *Clin Orthop* 1979; 144:326-35.
- Van Steensel CJ, Schreuder O, van den Bosch BF, et al.** Failure of anterior cruciate-ligament reconstruction using tendon xenografts. *J Bone Joint Surg [Am]* 1987; 69-A:860-4.