JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 5 (1994) 88–95

The regeneration of articular cartilage using a new polymer system

S. DOWNES, R. S. ARCHER, M. V. KAYSER
IRC in Biomedical Materials, Institute of Orthopaedics, Royal National Orthopaedic Hospital Trust, Stanmore, Middlesex, HA7 4LP, UK

M. P. PATEL, M. BRADEN
Department of Biomaterials in Relation to Dentistry, London Hospital Medical College, Turner Street, London, E1 2AD, UK

A polymer system based on room temperature polymerising poly(ethylmethacrylate) polymer powder and tetrahydrofurfuryl monomer has been investigated as a biomaterial for encouraging articular cartilage repair. This heterocyclic methacrylate polymer system swells slightly *in situ* and thus provides a good interface with subchondral bone resulting in mechanical stability with favourable uptake kinetics. Another feature of this polymer system is that it exhibits high water uptake which leads to absorption of the surrounding tissue fluid and matrix proteins, including growth factors; this may encourage the formation of new cartilage. Three weeks after implantation the tissue overgrowth contained cartilage components: chondrocytes, collagen type II, chondroitin 4-sulphate and chondroitin 6-sulphate. In addition numerous chondrocyte clones were observed at the edge of the defect and in the newly repaired tissue. By six weeks a superficial articulating surface was continuous with the normal articular cartilage with underlying tissue which showed some evidence of endochondral ossification. By nine weeks the surface covering of new cartilage had a widened and an irregular zone of calcified cartilage with thickened subchondral bone was present. At eight months the resurfaced cartilage remained intact above a remodelled subchondral bone end plate.

1. Introduction

Total joint replacement is not a satisfactory procedure for the younger patient because of the need for revision surgery due to loosening of the implant; a better procedure would be one that induced cartilage repair to give a normal functional articular surface. However the repair of defects in the articular cartilage has proved difficult [1–4]. Experimentally, there appear to be two categories of injury to cartilage one in which only the articular cartilage is broken and a second in which the subchondral bone is disrupted. Lesions that are restricted to cartilage do not heal [5–7] but, if the defect extends into the subchondral bone (full-thickness injuries), it undergoes a repair process. This undoubtedly involves bone marrow and blood vessels which participate in an inflammatory response and tissue remodelling [3, 6].

Biomaterials scientists continue to search for a material that could be used for cartilage repair. Investigators have filled the cartilage defect with a variety of natural and synthetic scaffolding materials, ranging from reconstructed collagen sponges [8] to carbon fibre implants [9] and polylactic acid (PLA) and periosteal grafts [10, 11], all intended to encourage the resurfacing of the defective region with a cartilagenous matrix.

In our study, the osteochondral defect was filled with a room temperature polymerizing system based on poly(ethyl)methacrylate polymer powder and tetrahydrofurfuryl monomer. An interesting and important feature of this polymer is that it exhibits high water absorption (up to 34%) *in vitro* [12], furthermore, unlike conventional xerogels, the uptake is protracted samples not reaching equilibrium in several months. Hence the kinetics of water uptake may be as important as the amount absorbed. *In vivo* this polymer should absorb tissue fluids *in situ* and hence gain similar biological properties to those of the host tissue and cells; this may create an environment which encourages cartilage overgrowth. The high water content of this polymer is similar to that of hyaline cartilage [13, 14], the natural tissue which must be encouraged to repair. The characterization of early tissue components growing over the novel polymer allows an evaluation of the biocompatibility of this polymer system and its potential for use in articular cartilage resurfacing.

2. Materials and methods

2.1. Polymer preparation

Poly(ethyl)methacrylate powder (5 g) and tetrahydrofurfuryl monomer containing 2.5% v/v N,N dimethyl-

p-toluidine (2.5 ml) were mixed for 1 min at room temperature with a sterile spatula. The resulting paste was inserted into a sterile 1 ml syringe.

2.2. Animal model

Eighteen mature Sandy-lop rabbits of at least 3.5 kg body weight were anaesthetised by injection with methohexitone sodium (Brietal) and maintained under anaesthestic with halothane (nitrous oxide, N_2O) and oxygen. Access to the femoral condyle was gained through a medial parapatellar capsulotomy. Dislocation of the patella enabled a single 3 mm diameter full depth osteochondral defect to be drilled by hand above the intercondylar notch. Drill holes were washed with sterile saline to remove debris and 0.15 ml of pre-polymer was inserted from a sterile syringe into the subchondral bone.

Polymerization occurred *in situ*. The patella was relocated and the wounds were closed with sutures (Dexon *Plus, Cyanamid). The rabbits were group housed in floor pens [15] and thus had freedom of movement. All rabbits recovered from the surgery and were moving, eating and drinking within 24 h. Rabbits were sacrificed at 3, 6, 9, 12 weeks (four in each group) and 8 months (two rabbits) after surgery by intravenous injection of pentobarbitone sodium. The articular surface of the femoral condyles, particularly the region of defect repair, were examined macroscopically.

2.3. Histology and immunolocalization

Excess bone was removed from the femoral condyles before fixation in 2% paraformaldehyde and 0.5% glutaraldehyde in 0.02 M phosphate buffer, at 4 °C, for 48 h. Specimens were decalcified in neutral EDTA at 4 °C until mineral could be no longer detected by radiography. Specimens were then washed in 0.02 M phosphate buffer overnight prior to tissue processing. The pairs of femoral condyles from the rabbits sacrificed at each time point were processed for cryostat sectioning, wax sectioning or electron microscopy.

2.4. Cryostat sectioning

Femoral condyles were mounted on a cork disc in cryomountant (Cryo-M-Bed, Brights Instrument Co.) and frozen by immersion in liquid nitrogen (British Oxygen Company), before cryostat sectioning at − 20 °C on to double-sided sellotape. The sellotape held the brittle polymer in place and intact sections were then stuck to glass slides and stored at − 70 °C for immunolocalization studies or stained briefly with methylene blue-azur II, before mounting with aquamount (Merck Ltd) under glass coverlips. Sections were photographed immediately.

2.5. Wax sectioning

Femoral condyles were dehydrated through a graded series of ethanols (70%, 90%, 100%) into xylene at room temperature over 2 h with two changes of xylene. During this time softened polymer was carefully removed through the bone from below the repaired defect to prevent expansion of the polymer through the region of repair. Xylene/wax (1:1) impregnation continued for 30 min at 60 °C and wax impregnation at 60 °C for 2 h before wax embedding and storage at 4 °C.

2.6. Immunolocalization of collagen type II, chondroitin 4-sulphate and chondroitin 6-sulphate

Cryostat sections or sections were dewaxed and rehydrated into 100 mM tris buffer pH 7.2 containing 150 mM NaCl and 0.5% bovine serum albumin (BSA, Sigma Chemical Co.) They were digested with chondroitinase ABC (0.5 I.U./ml, Sigma Chemical Co.) for one hour at 37 °C to reveal the epitopes before immunolocalization. Sections were washed three times in the same Tris buffer after pre-digestion and between each immunolocalization step; this buffer was also used for dilution of primary and secondary antibodies. Selected primary monoclonal antibodies were applied individually for 45 min, in a dark humidified atmosphere at room temperature, before a rhodamine conjugated anti-murine serum (1/40 dilution, Dako Ltd.) was applied to each section for 45 min under the same conditions. Non-immune mouse serum was applied to control sections for all immunolocalizations and a pre-adsorbed anti-collagen type II monoclonal antibody was used as an extra control for the localization of collagen type II. Sections were mounted under glass cover slips in glycerol/PBS (9:1) containing 1,4-diazobicyclo [2.2.2.] octane, (DABCO, 25 mg/ml, Sigma Chemical Co.) to retard photobleaching [16].

2.7. Primary antibody details

Collagen type II. Primary monoclonal antibody CIICI (Developmental Hybridoma Bank, University of Iowa, USA.) [17] was diluted 1:1.

Chondroitin 4-sulphate (2-*B*-6) *and Chondroitin 6-sulphate* (3-*B*-3). Primary monoclonal antibodies 2-B-6 and 3-B-3 (Kennedy Institute of Rheumatology, London, UK) [18] were diluted 1:100.

2.8. Electron microscopy

The rabbits were sacrificed and the femora and adherent tissue removed before the femoral condyles were cut either side of the drill hole filled with cement. The block of tissue was fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2 for 24 h at 4 °C followed by secondary fixation in an aqueous solution of 1% osmium tetroxide and 1.5% potassium ferrocyanide [19–22] for 1 h. The sample was washed in the same buffer and dehydrated through a graded series of ethanols (70%, 90%, 100%). The cement was carefully removed without damaging the surface cartilage. Impregnation with a 1:1 ethanol/Spurrs' resin mixture for 6 h was then carried out including 2 h vacuum impregnation at 15 kPa. The resin was made up as follows: ERL4206 (20 g), DER736 (8 g), NSA

(52 g), S-1 (0.4 g), mixed well, sealed in air-tight containers and stored at −20 °C until needed. The 1:1 mixture of ethanol/resin was followed by four changes of 12 h each of Spurrs' resin alternating every 6 h with vacuum infiltration. The specimens were embedded and cured at 70 °C for 18 h. One micrometre sections were cut with a diamond knife for light microscopy (LM) and stained with methylene blue-azur II-basic fuchsin stain [23]. Selected areas for transmission electron microscopy (TEM) were cut on a L.K.B. Ultratome III. The sections were stained with 2% uranyl acetate (10 min) and Reynold's lead citrate (10 min) [22].

3. Results

3.1. Macroscopic findings

None of the rabbits died, nor was there any evidence of infection. The rabbits were housed in group pens which allowed freedom of movement, i.e. running, jumping, standing on hind legs. The animals showed no sign of discomfort and all enjoyed full mobility. In most rabbits the knee joints showed a white glistening cartilage-like tissue resembling the normal surrounding articular cartilage. There appeared to be a good overgrowth of cartilage over the polymer within the defect. However, in three rabbit knees the tissue covering was incomplete; histological observations revealed that the polymer had been set *above* the level of the subchondral bone in the cartilage defect. We now realize that, since cartilage cannot grow through the polymer, the polymer must be set at the appropriate level to allow good resurfacing (Fig. 1).

3.2. Bone–polymer interface

The frozen sections allowed visualization of the intact polymer–bone interface. There was good integration between the subchondral bone and the polymer. Viable osteoblasts and dense collagen were observed at the polymer interface.

3.3. Resurfaced cartilage

3.3.1. 3 weeks

At 3 weeks a new tissue layer had formed over the polymer surface; this appeared to be composed of some fibrous and some cartilaginous tissue (Fig. 2). The surface of the cartilage appeared to be composed of dense fibrous tissue containing many cells with the lateral orientation of fibroblasts. In deeper regions there were areas in which the cells appeared more rounded in a less dense matrix; some chondrocytes were seen in lacunae and were separated by an increased amount of matrix. Immunolocalization studies showed that, in areas where the cells were more rounded and appeared chondrocytic, there was coincident production of collagen type II (Fig. 3a and 3b). The collagen type II appeared to form an even matrix around the chondrocytes but was not present in the fibrous regions. Chondroitin 4-sulphate appeared throughout the new tissue layer; both in fibrous areas and the more cartilagenous regions (Fig. 4a and 4b). Chondroitin 6-sulphate was also present but not throughout the tissue layer: it appeared to be produced by cells immediately above the polymer (Fig. 5a and 5b). Another feature observed by light and electron microscopy was chondrocyte clones. These were usually found at the lateral margin of the original drill hole where the polymer interfaced with the superficial subchondral bone (Fig. 6). Numerous individual cells were observed within an easily defined pericellular "basket". The chondrocytes within the clones were rounded, and characteristically contained large quantities of endoplasmic reticulum and glycogen stores within the cytoplasm (Fig. 7). The cell surfaces exhibited numerous short processes (Fig. 6 insert).

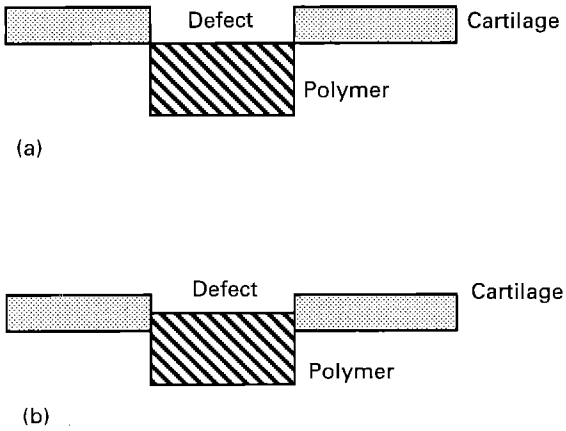

Figure 1 The polymer should be set at the same level as the subchondral bone (a) and not across the cartilage defect (b)

3.3.2. 6 weeks

The defect was completely filled with dense tissue. Fibrous tissue was evident in the superficial layer with areas of chondrogenesis in the deeper regions. Fig. 8 shows a cryosection of the resurfaced cartilage at six weeks; the sections have been attached to double-sided cellotape to keep the polymer in place. Within the surface layer of fibrous tissue there were regions of rounded cells and the tissue appeared more similar to hyaline cartilage. Fragments of the polymer were observed in the new tissue (p), this was attributed to

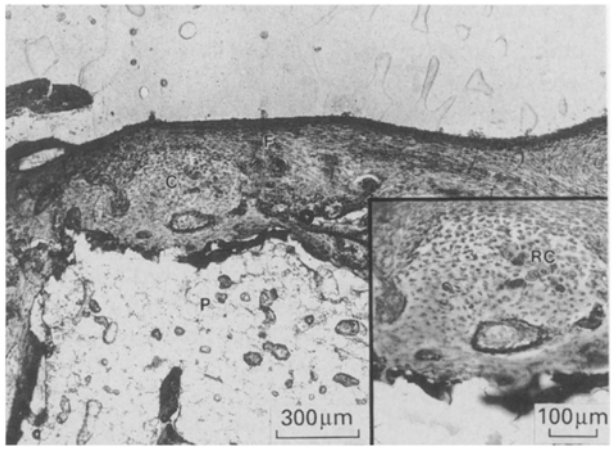

Figure 2 Cryostat section stained with toludine blue. Notice the highly cellular resurfaced cartilage 3 weeks after implantation of the polymer. The surface appears fibrous (F), deeper regions show a more cartilaginous tissue (C) with chondrocytes in lacunae (RC). P shows the polymer.

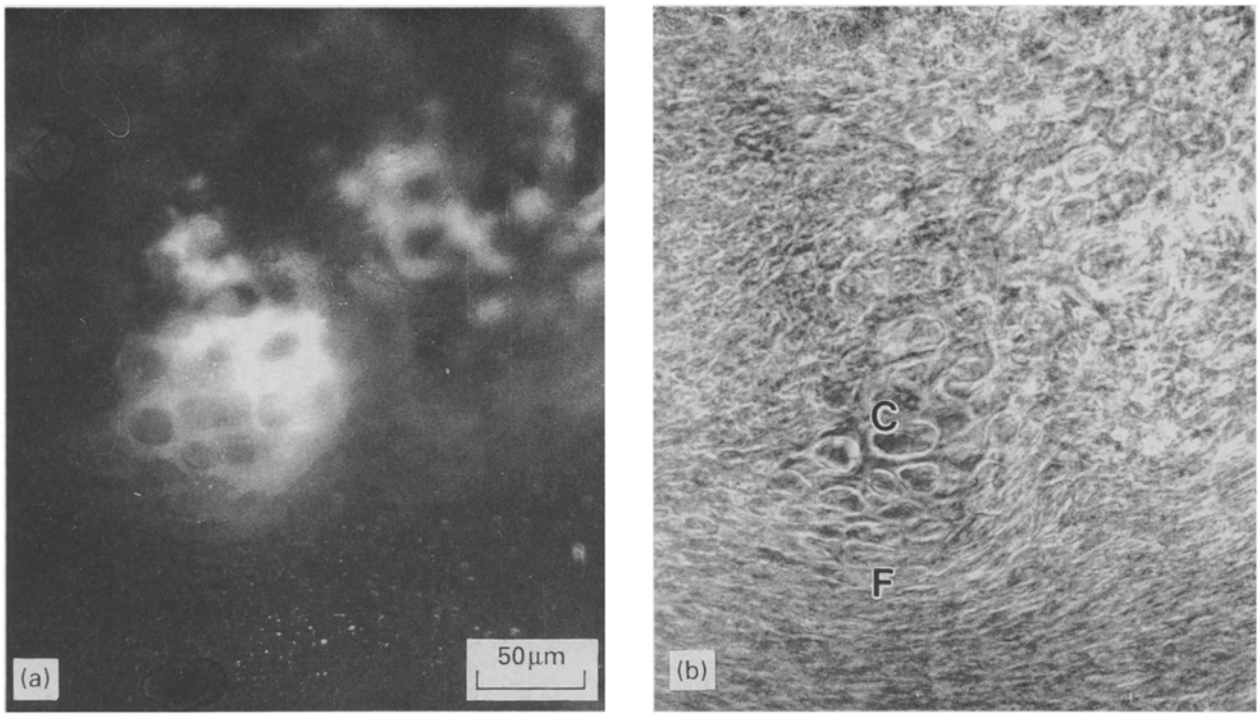

Figure 3 Immunolocalization of collagen type II in resurfaced cartilage at 3 weeks: (a) immunfluoroscence; (b) phase photograph. F = fibrous tissue, C = cartilaginous tissue. Note that the collagen II occurs only around the chondrocytes.

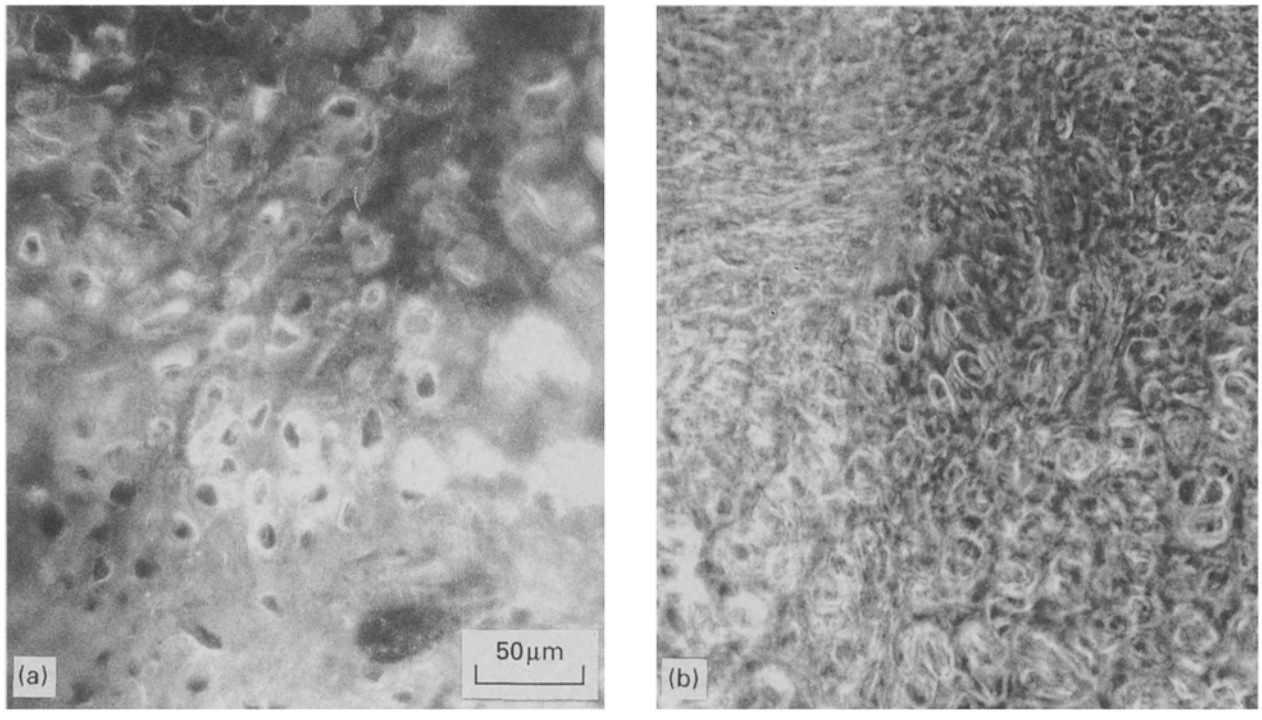

Figure 4 Immunolocalization of chondroitin-4-sulphate in resurfaced cartilage at 3 weeks: (a) immunfluoroscence; (b) phase photograph. Immunofluoroscence occurs throughout the matrix.

surgical technique and in later studies the polymer surface was flattened using a rounded instrument before closure of the joint. Immunolocalization studies demonstrated that collagen type II, chondoitin 4-sulphate and chondroitin 6-sulphate were present in the tissue. Collagen type II was found in regions where cells appeared rounded; it was not found in the fibrous tissue. In some sections there were areas of endochondral ossification below the cartilage but above the polymer. Bone adjacent to the medullary defect showed signs of creeping substitution, the juxtaposition of new bone on pre-existing trabecular bone.

3.3.3. 9 weeks

By 9 weeks there was more evidence of remodelling of the subchondral bone. The cartilage above the bone

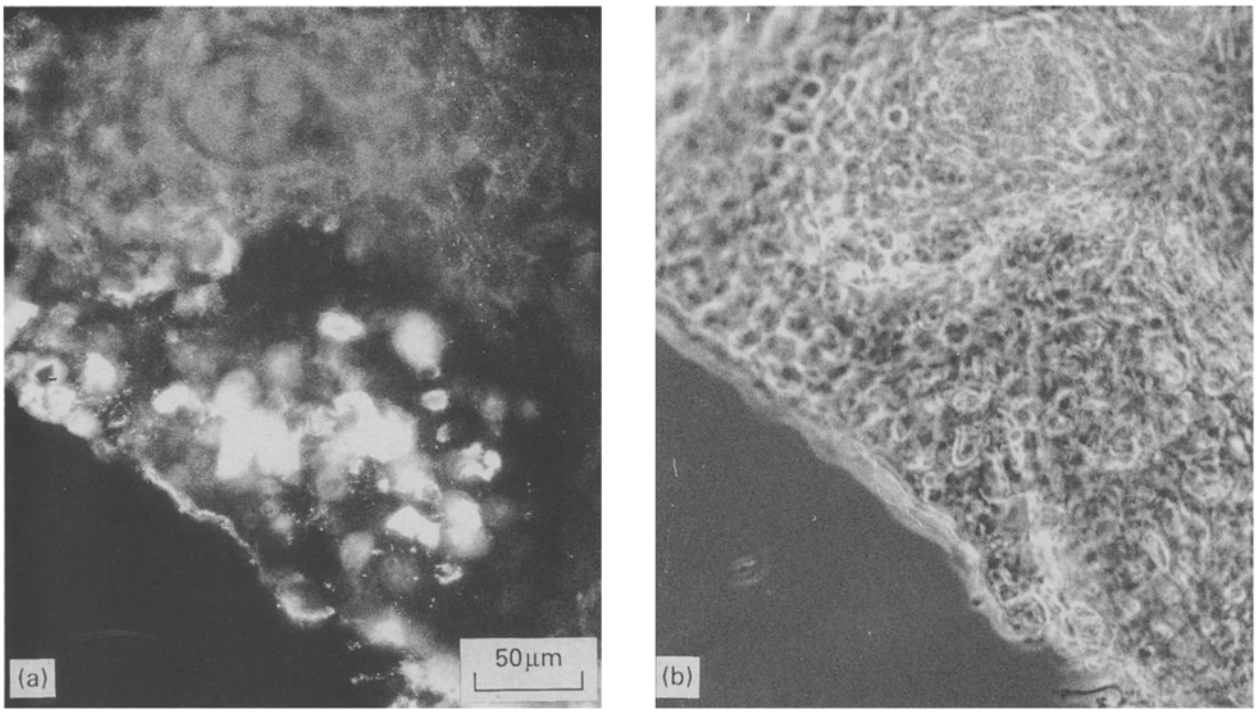

Figure 5 Immunolocalization of chondroitin-6-sulphate in resurfaced cartilage at 3 weeks: (a) immunfluoroscence, (b) phase photograph. Immunofluoroscence occurs in the regions adjacent to the polymer. P = polymer.

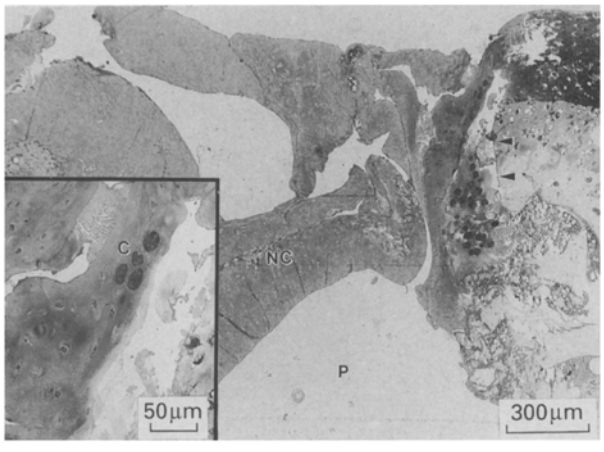

Figure 6 Histology of the defect at 3 weeks. Epoxy resin section stained with methylene blue-azure II-Basic fuchsin stain. Notice the appearance of the resurfaced cartilage which appears to be growing from the original cartilage. Arrows mark the edges of the defect, P = polymer space and NC = new cartilage. C denotes the chondron clones at the edge of the defect at higher magnification.

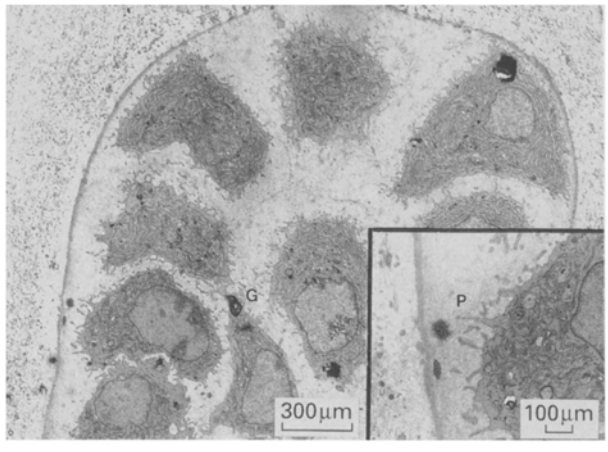

Figure 7 An electronmicrograph of a chondron clone. Notice the glycogen stores (G) and the fine processes (P) radiating from the chondrocytes.

remained intact with many cells present but within a less dense matrix than the original cartilage. There were chondrogenic regions in both the new bone and beneath the new cartilage. Fig. 9 shows a wax embedded section of the resurfaced defect; the processing for wax embedding involves the removal of the polymer, although there were traces of polymer (p) left in the section. Immunolocalization of the matrix components in the 9 week wax embedded tissue sections confirmed the presence of collagen type II in the cartilage layer of new tissue. Chondroitin 4-sulphate was associated with chondrocytes within the new

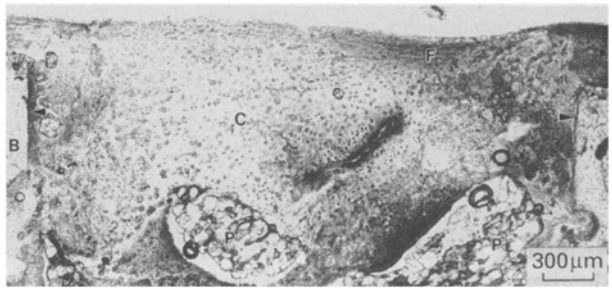

Figure 8 A cryostat section of the defect with polymer after 6 weeks. Arrows mark the edge of the defect, F = fibrous tissue, C = chondrogenic regions, B = bone and P = polymer.

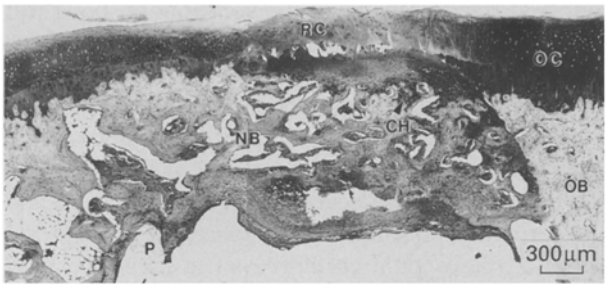

Figure 9 A wax embedded section of the defect after 9 weeks. OC = original cartilage, RC = resurfaced cartilage. Note the chondrogenic regions (CH). The subchondral bone has remodelled as shown by the new bone formed (NB), OB = original bone. During the processing the polymer was removed but had occupied the space below the new bone, a small amount of polymer (P) remains.

tissue and sparsely distributed throughout the more fibrous tissue. Chondroitin 6-sulphate was also associated with chondrocytes but was also strongly associated with the new fibrous tissue.

3.3.4. 8 months

Two rabbits were kept for a longer study (8 months). The joints remained functional throughout the study period. The histology (Fig. 10) revealed that the new cartilage remained intact but the density of the matrix had still not achieved that of the original cartilage. There were still a mixed population of cells and areas of fibrous and chondrogenic regions. The subchondral bone had remodelled and in it the polymer became surrounded by very dense collagen.

3.4. Collagen

A comparison was made of the ultrastructure of the collagen in both the new cartilage and the bone surrounding the polymer. Fig. 11 shows the broad-banding collagen (type II) in the resurfaced cartilage after eight months; the collagen fibrils can be seen in both transverse and longitudinal section. Fig. 12 shows the narrow-banding collagen (type I) observed adjacent to the polymer in the bone. The classical mineralization of the osteoid can be seen with the deposition of hydroxyapatite in the collagenous matrix.

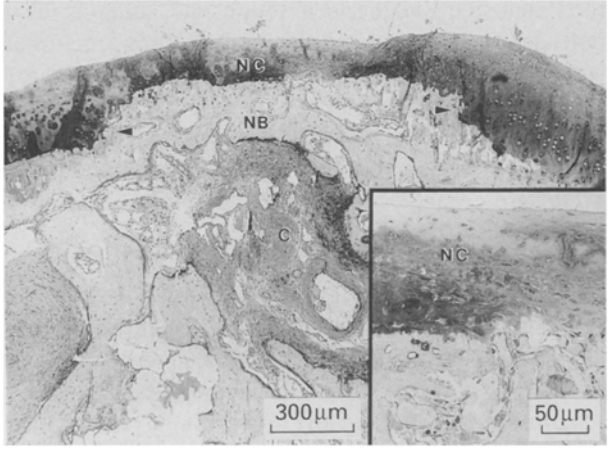

Figure 10 A resin embedded section at 8 months. The arrows indicate the edge of the defect, the remodelled subchondral bone (NB) can be seen with dense collagen (C) surrounding the polymer. The new cartilage (NC) remains intact but still a mixed population of cells.

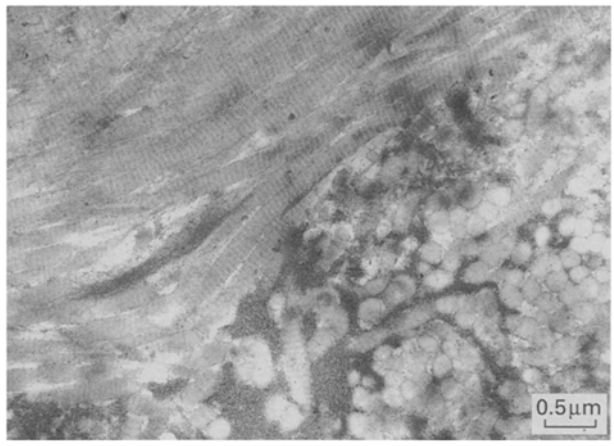

Figure 11 A transmission electronmicrograph of collagen (type II) observed in the resurfaced cartilage at 8 months.

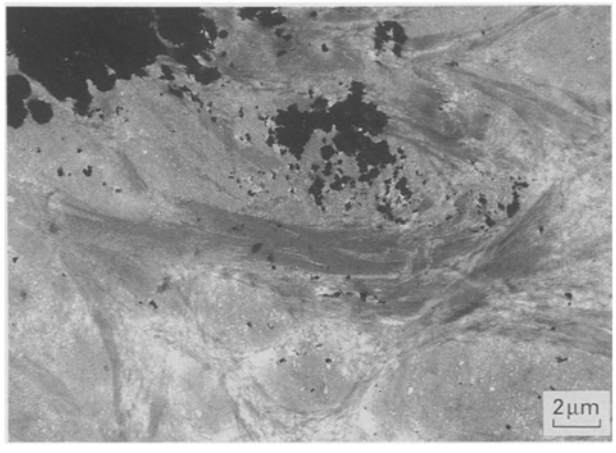

Figure 12 A transmission electronmicrograph of collagen (type I) observed in the remodelled bone at 8 months. Note the foci of hydroxyapatite crystals leading to mineralization of the collagen.

3.5. Controls

In six random cases the defect was not filled with polymer but left as a hole. In these cases there was a mixed cell response with areas of chondrogenesis, areas of active fibroblasts and areas of bone remodelling. In general there was no significant repair of cartilage (Fig. 13).

4. Discussion

Buckwalter [2, 3] has shown that the ability of cartilage to repair is variable and depends upon the type of insult inflicted on the cartilage. Injuries which cause a defect above the zone of calcified cartilage do not repair [3, 5–7], whereas defects which extend into the subchondral bone tend to repair; this is thought to be due to the presence of a blood supply and bone

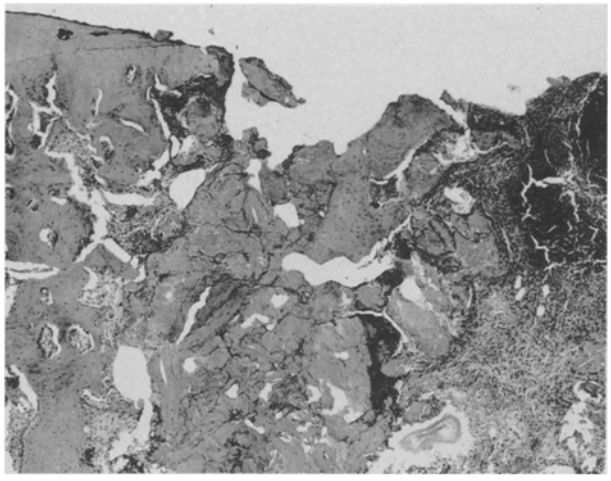

Figure 13 A resin embedded section of a control knee. Note the dissorganized repair showing degeneration of the subchondral bone and areas of fibroblast and chondrocyte regeneration.

marrow cells which are involved in the tissue remodelling [3]. Bone matrix contains many growth factors that can stimulate chondrogenesis [24–28]; the disruption of the bone matrix will release these factors which may influence both cartilage and bone repair in the osteochondral defect. An important property of this polymer system is that it exhibits high water uptake *in vitro* [12]; assuming that this property is maintained *in vivo* then the polymer will absorb tissue fluids and growth factors from the bone matrix, bone marrow and synovial fluid, which may create an advantageous environment to encourage cartilage overgrowth. There are a number of other important features of this polymer. The material is not biodegradable and remains intact in the bone, the surface of the polymer is smooth [29] and there is little chance of particulate debris at the surface which could cause wear problems. The material swells due to water uptake after polymerization hence, *in situ*, a tight bond occurs between the polymer and the bone, providing a mechanically stable implant which is clearly important for the formation of a functional joint. The polymer is fairly hydrophilic but the water uptake process as stated earlier is protracted. Since natural cartilage is composed of 60% to 80% water, we believe that a hydrophilic polymer has advantages when used in cartilage repair. Hydration of cartilage is essential because chondrocytes rely upon diffusion for the supply of nutrients rather than a vascular supply. The light and electron microscopical studies of both the cartilage and the bone showed very dense collagen; the reasons for this are unclear but may be due to the hydrophilicity of the polymer. During the study we realized that it was important to set at the right level within the bone, i.e. slightly below the surface of the subchondral bone; if the polymer was too high the surface was not completely covered with a new cartilage layer, while if it was too low, bone surrounded the polymer and cartilage repair did not occur.

There were three biological boundaries to the area of defect, namely bone, synovium and articular cartilage. There are various methods by which the formation of new cartilage and bone could occur. We suggest that the disruption of the subchondral bone will allow marrow cell migration providing a cellular source for chondrocyte differentiation [30, 31], also the disruption of blood vessels would promote new bone formation. Similarly synovial fluid may provide chondrogenic cells, fibroblasts and growth factors. There was also an indication of cell proliferation and the formation of chondrocyte clones at the severed edges of the original cartilage, hence causing expansion of the old cartilage over the new polymer. We consider the presence of chondrocyte clones in the new cartilage to be highly relevant. Chondrons are considered to be a primary metabolic unit in hyaline cartilages (32, 33). Poole (36) has described chondrons in articular cartilage composed of a chondrocyte, its pericellular matrix and a compacted filamentous capsule thought to contain minor collagens (34, 35). Poole postulates that the chondron acts hydrodynamically to protect the chondrocyte during compressive loading. They have also been reported in cartilage during the early stages of osteoarthritis (37–39) and they are thought to be engaged in matrix synthesis or possibly destruction (40). It is interesting to note that clones reported in articular cartilage generally have the appearance of multiple chondron columns (41, 42) whereas in this study the clones appeared rounded and contained between four and ten chondrocytes. There has been other reports of chondron clones in the regeneration of articular cartilage defects after both chondral shaving and subchondral abrasion (43); these chondrons were also multi-cellular with a rounded appearance. We suggest that chrondrons are important for the regeneration of hyaline cartilage in an articular defect although the exact role is unclear. We regard this new polymer system as a good biomaterial for the repair of articular cartilage defects. As early as 3 weeks after surgery there was a complete covering of new tissue which appeared to be a mixture of cartilage and fibrous tissue which, with time, became more cartilagenous. The presence of chondrocytes and chondrocyte clones with the immunolocalization of collagen type II, chondroitin 4-sulphate and chondroitin 6-sulphate are good indicators that the resurfaced tissue resembles hyaline cartilage. We have seen that the subchondral bone end plate is also remodelled with time and the joint remains functional with intact cartilage above new subchondral bone for at least 8 months and we suggest that this would continue for a much longer time period. These experiments have been done using an animal model but we have no reason to doubt that a similar procedure will be successful in human joints.

5. Conclusion

The polymer system used in this investigation allows the resurfacing of full thickness articular cartilage defects in an animal model. The repair tissue contained chondrocytes, collagen II and proteoglycan species normally produced by chondrocytes.

Acknowledgement

We would like to thank Gary Batchelor for his help with the animal work.

References

1. H. S. CHEUNG, K. L. LYNCH, R. P. JOHNSON and B. J. BREWER, *Arth. Rheum.* **23** (1980) 211–19.
2. J. A. BUCKWALTER and V. C. MOW, in "Osteoarthritis: diagnosis and management", 2nd Edn, edited by D. S. Howell, H. J. Mankin, R. W. Moskowitz and W. B. Saunders, (Philadelphia 1990).
3. J. A. BUCKWALTER, L. C. ROSENBERG and E. B. HUNZIKER, in "Atricular cartilage and knee joint function: basic science and arthroscopy, edited by J. W. Ewing, (Raven Press, New York, 1990) Ch. 2.
4. T. FURUKAWA, D. R. EYRE, S. KOIDE and M. GLIMCHER, *J. Bone Jt. Surg.* *A***62** (1980) 179–89.
5. N. MITCHELL and N. SHEPHARD, *J. Orthop. Res.* **5** (1987) 388–92.
6. G. MEACHIM, *J. Bone Jt. Surg.* *B***45** (1963) 150–61.
7. H. J. MANKIN and C. H. BOYLE, in "Cartilage degradation and repair" edited by A. L. Bassett (National Academy of Science, National Research Council, Washington, DC, 1967) pp. 186–199.
8. D. P. SPEER, M. CHAVPIL, R. G. VOLZ and M. D. HOLMES, *Clin. Orthop.* **144** (1979) 326–35.
9. R. J. MINNS, D. S. MUCKLE and J. E. DONKIN, *Biomaterials* **3** (1982) 81–6.
10. H. P. VON SCHROEDER, M. KWAN, D. AMIEL and R. D. COUTTS, *J. Biomed. Mater. Res.* **25** (1991) 329–39.
11. J. M. RUBAK, *Acta Orthop. Scand.* **53** (1982) 1750–80.
12. M. P. PATEL and M. BRADEN, *Biomaterials* **12** (1991) 645–58.
13. F. F. JAFFE, H. J. MANKIN, C. WEISS and A. ZARINS, *J. Bone Jt. Surg.* *A***56** (1974) 1031–9.
14. F. C. LINN and L. SOKOLOFF, *Arthritis and Rheum.* **8** (1965) 481–94.
15. G. R. BATCHELOR, *Animal Technol.* **42**(2) (1991) 109–20.
16. G. D. JOHNSON, R. S. DAVIDSON, K. C. McNAMEE, G. RUSSELL, D. GOODWIN and E. J. HALBARROW, *J. Immuno. Methods* **55** (1982) 231–42.
17. R. HOLMDAHL, K. RUBIN, L. KLARESHOG, E. LARSSON and H. WIGZELL, *Arthritis Rheum.* **29** (1986) 400–10.
18. B. CATERSON, J. E. CHRISTNER, J. R. BAKER and J. R. CROUCHMAN, *Fed. Proc. Fedn. Amer. Soc. Exp. Biol.* **44** (1985) 386–93.
19. W. C. DE BRUIJN, *J. Ultrastructural Res.* **42** (1973) 29–50.
20. A. M. DVORAK, *Lab Investigation* **27**(6) (1972) 561–4.
21. C. E. FARNUM and N. J. WILSMAN, *J. Histochem. Cytochem.* **31** (1983) 765–75.
22. M. J. KARNOVSKY, American Society for Cell Biology, 11th Annual Meeting, New Orleans (1971)
23. C. D. HUMPHREY and F. E. PITTMAN, *Stain Technol.* **49** (1974) 9–14.
24. A. H. REDDI and C. HUBBINS, *Proc. Natl Acad. Sci. USA* **69** (1972) 1601–1605.
25. A. H. REDDI, in "Degerative joints", Vol. 2 edited by G. Verbrugger and E. M. Veys, (Excerpta Medica, Amsterdam, 1985).
26. *Idem., Rheumatol.* **10**(11) (1983) 67–9.
27. A. H. REDDI, S. WEINTROUB and N. MUTHUKUMARAN, *Orthop. Clin. North Amer.* **18** (1987) 207–12.
28. T. K. SAMPATH, D. P. DeSIMONE and A. H. REDDI, *Exp. Cell Res.* **142** (1987) 460–2.
29. S. DOWNES, M. P. PATEL, K. DAVY and M. BRADEN, Presented at Surface Properties of Biomaterials, International Symposium, May 1992, Manchester, UK, in press.
30. S. M. SEYEDIN, A. Y. THOMPSON, H. BENTZ, D. M. ROSEN, J. M. McPHERSON, A. CONTI, N. R. SIEGEL, G. R. GELLUPPI and K. A. PIEZ, *J. Biol. Chem.* **261** (1986) 5693–5.
31. M. B. SPORN, A. ROBERTS, L. M. WAKEFIELD and R. K. ASSOIAN, *Science* **233** (1986) 532–4.
32. J. A. SZIRMAI, in "Aging of connective and skeletal tissue" edited by A. Engel and T. Carsson, Thule Institute Symposium, Nordiska bokhandelns, Stockholm, 1969 pp. 163–84.
33. R. WOBST, G. HOMMEL and K. SAMES, *Gerontology (Basel)* **26** (1980) 311–20.
34. C. A. POOLE, M. H. FLINT and B. W. BEAUMONT, *J. Anat.* **138** (1984) 113–38.
35. *Idem., J. Ultrastructure. Res.* **91** (1985) 13–23.
36. *Idem., J. Orthopaedic Res.* **5** (1987) 509–22.
37. D. H. COLLINS and T. F. McELLIGOTT, *Ann. Rheum. Dis.* **19** (1960) 318–30.
38. G. MEACHIM and D. H. COLLINS, *ibid.*, **21** (1962) 45–50.
39. H. J. MANKIN, in Adult orthopaedics edited by R. L. Cruess and W. Rennie (Churchstone Livingstone New York, 1984) pp. 163–270.
40. N. MITCHELL, E. R. LEE and N. SHEPHARD, *J. Bone Jt. Surg.* *B***74** (1992) 33–8.
41. C. A. POOLE, S. AYAD and J. R. SCHOFIELD, *J. Cell Sci.* **90** (1988) 635–43.
42. C. A. POOLE, T. HONDA, S. J. M. SKINNER, J. R. SCHOFIELD, K. F. HYDE and H. SHINKAI, *Connective Tiss. Res.* **24** (1990) 319–30.
43. H. K. W. KIM, M. K. E. MORAN and R. B. SALTER, *J. Bone Jt. Surg.* *A***73** (1991) 1301–15.

Received 17 November 1992
and accepted 23 March 1993