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E-PTFE in rabbit knee-joints

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The healing of expanded polytetrafluoroethylene (e-PTFE) in articular cartilage and bone was studied. A 1 × 4 mm osteo-chondral defect was created in the medial femoral condyle in 10 rabbits (20 knee-joints). A correspondingly broad strip of e-PTFE was placed in the defects and pulled through two drilled channels to the dorso-lateral side of the condyle. The contra-lateral knee-joint served as control. The animals were not immobilized and allowed to move about freely together in a room. The animals were killed by perfusion fixation after 14 months, the implants and tissues retrieved en bloc and examined with scanning electron microscopy (SEM) and light microscopic (LM) morphometry. No macroscopic signs of inflammation were detected in the knee-joints. Observations with SEM in control joints showed that the articular surface ranged from smooth to irregular with superficial crevices and fibrillations at the site of the defect. The smooth articular surface of the surrounding articular cartilage partly overlapped the e-PTFE membrane. The surface of the e-PTFE membrane had a nodular character and was surrounded by fibrocartilage with clusters of chondrocytes. A consistent observation was the large amount of bone around and in direct contact with the surface of e-PTFE membrane. LM morphometry of intact e-PTFE-tissue specimens in three different section planes showed that 73.1% and 8.8% of the implant surface was in contact with bone and bone marrow, respectively. Our morphological observations of e-PTFE in the cartilage and bone of the rabbit knee-joint after a 14-months healing period indicate that e-PTFE could be a useful material in reconstructive surgery of smaller non-weight-bearing joints.

1. Introduction

Resurfacing arthroplasties have been tried with different sorts of materials, biologic and synthetic. However, most materials have failed either by degeneration or by causing adverse tissue reactions [1–5]. It has been suggested that biochemical factors might cause inflammation and that wear particles from implants can trigger this process [6–8]. In an earlier short-term light microscopical study (12 weeks) it was shown that e-PTFE (expanded polytetrafluorethylene) integrated well with the articular cartilage in the rabbit medial femoral condyle. The joint facing surface of the membrane was covered by a multicellular fibrocartilage-like tissue layer [9].

E-PTFE is a polymer of repeating carbon-fluor units, arranged as nodes which are connected by flexible fibrils. The material is regarded as biochemically inert and is considered to have good biomechanical qualities [10]. The microporous structure of the material permits cell in-growth if the membrane is manufactured with large enough pores [9, 11,12]. E-PTFE is used in eye surgery for reconstruction of orbital floor defects [13, 14] and in dental surgery as a barrier to seal bone defects from surrounding tissues in order to exclude non-bone forming cells and promote bone regeneration [15, 16]. When e-PTFE is used for tissue repair in the body, it has been shown to cause a low degree of inflammation and adverse reactions [17–20]. However, the interaction between bone and e-PTFE surfaces has not, to our knowledge, been evaluated. The aim of the present study was to evaluate the possibility of restoring an articular surface in a long-term experiment. In addition the healing of e-PTFE in bone was examined.

2. Material and methods

2.1. Animals and anaesthesia

Twelve female New Zealand White rabbits weighing between 2.7 and 3.1 kg were used. The animals were operated on under general anaesthesia with 0.3–0.5 ml/kg Hypnorm® (Fluanison 1 mg ml^{-1} and Fentanyl 0.2 mg ml^{-1}) and 0.5 ml Rompun® (Xylazine chloride 20 mg ml^{-1}) given intramuscularly about 30 min preoperatively, supplemented with local administration of 10–20 mg Xylocain® (Lidocain hydrochloride 10 mg ml^{-1}). Penicillin (Intencillin®)

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375 000 IU was given once intramuscularly immediately before surgery.

2.2. Implant

One-millimetre-wide e-PTFE strips were created by cutting from commercially available sheaths of e-PTFE (e-PTFE, Gore-Tex® , W L Gore & Assoc., Inc, Flagstaff, USA) surgical membrane (thickness 1 mm; pore size 30 μm) (Fig. 1a,b).

2.3. Surgery and animal care

The knee joints were shaved with an electric shaver and scrubbed with iodine. Under aseptic conditions the knee joints were exposed through a medial parapatellar incision and the patella was luxated laterally. An osteochondral defect, about 1 × 4 mm, was created with a small osteotome in the most ventral part of the weight-bearing articular surface of the medial femoral condyle. The defect was made deep enough to achieve a bleeding surface in the subchondral bone. The defect was surrounded by intact cartilage. A 1 mm wide and 50 mm long e-PTFE strip was placed in the defect to restore the cartilage defect. The e-PTFE strip was pulled via two drilled channels (diameters 1 mm) at either end of the osteo-chondral defect through the condyle to the dorsolateral side of the condyle. The membrane was firmly secured by sutures (Fig. 2). In the contralateral control knee a similar defect was made but left untreated. The wound was closed in layers with interrupted 4-0 Dexon® (a polyglycolic acid). The animals were allowed to move about freely together in a room (2 × 6 m) for the duration of the experiment.

2.4. Implant retrieval and tissue processing

After 14 months the animals were anaesthetised as described above supplemented with small doses of sodium pentobarbital given intravenously. The animals were perfused with 2.5% glutaraldehyde in 0.05M cacodylate buffer, pH 7.4, via the left heart ventricle for 5 min after the vascular system had been thoroughly rinsed with saline (0.9%). The entire knee joint was removed and immersed in glutaraldehyde. The fixated knee joints were opened and the articular surfaces examined and photographically documented using a Leitz dissection microscope. Specimens from the synovial tissue on the lateral femoral condyle were retrieved. Thereafter, the medial and lateral femoral condyles and tibial plateau's were divided by sawing (using dental equipment) under generous saline irrigation. The specimens were further immersed in 2.5% glutaraldehyde, dehydrated in ethanol and embedded in plastic resin (LR White) (eight of the ten rabbits). Ground sections (approximately 10 μm thick) of the

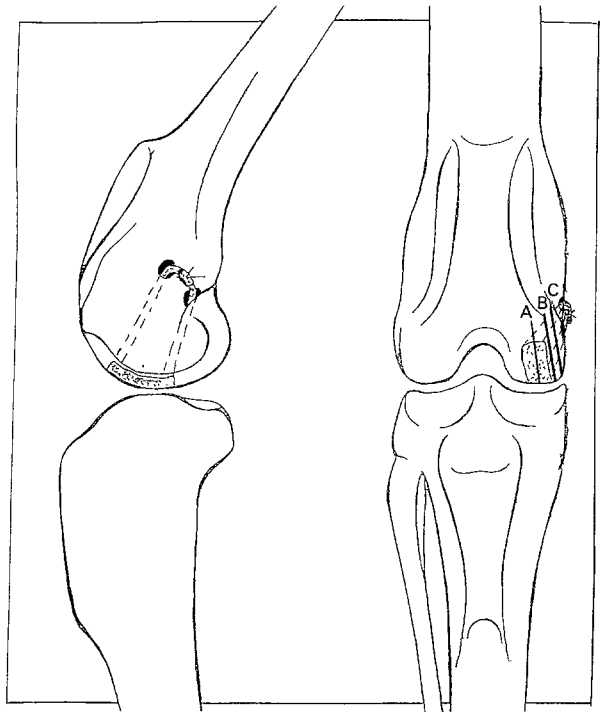
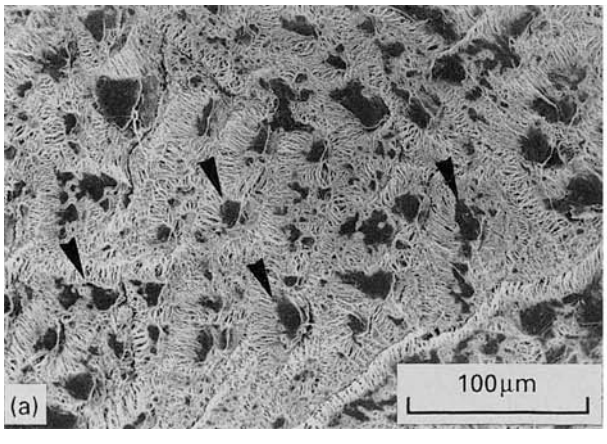


Figure 2 Schematic drawing of the operative technique for inserting the e-PTFE implant into the medial femoral condyle of the rabbit joint. The section planes (A, B and C) for preparing the histological specimens are indicated.



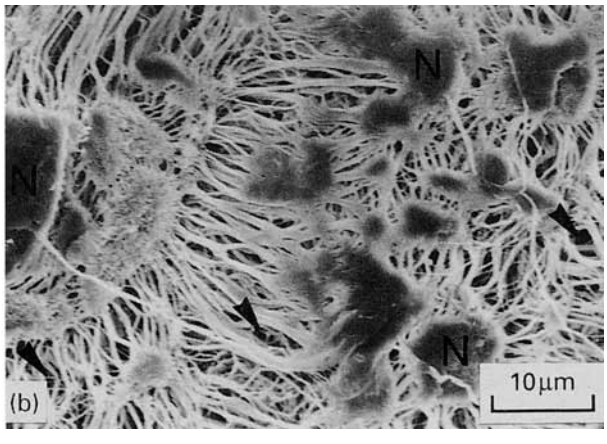


Figure 1 (a) Scanning electron micrograph of an e-PTFE sheath. The sheath consists of nodules (some of which are indicated by arrow-heads) interconnected by numerous fibrils. (b) Scanning electron micrograph of an e-PTFE sheath. The nodules (N) have varying sizes, shapes and internodular distance (also observed in Fig. 1a). Openings or pores (some of which are indicated by arrow-heads) are observed between the fibrils.

embedded implants, were cut for light microscopy and morphometry [21]. Sections were obtained from three different locations along the course of the implant: the part facing the joint and subchondral areas (section A), the mid-condylar part (section B) and the lateral-condylar part (section C) of the condyle (Fig. 2). Ground sections from the contra-lateral control joints were obtained by sawing through the site of the earlier created cartilage defect in the medial condyle. Ground sections were also prepared from the whole tibial plateau of three joints with implants. The ground sections were stained with 1% toluidine blue. For the morphometrical evaluation a Leitz Metallux microscope equipped with a Leitz Microvid connected to an IBM PC was used. On each section the total circumference (μm) of the e-PTFE implant and the distances (μm) of the implant surface in contact with different tissues (bone, bone marrow, loose connective tissue, dense connective tissue and inflammatory tissue) were measured. The data are given as the percentage of implant–tissue contact.

The synovial tissue specimens were embedded in epoxy resin (Agar 100, Agar Aids, Stansted, Essex, England), semithin (about 1 μm thick) sections cut with glass knives and stained with Richardson's solution (0.5% Azur II and 0.5% methylene blue in 1% disodium tetraborate).

Specimens from the surfaces of experimental and control joints in two of the ten rabbits were examined by scanning electron microscopy (Jeol T-300).

3. Results

Two animals had to be excluded. One rabbit was sacrificed due to an infection in the scapular region and one animal died of unknown cause after 6 months. The material thus consisted of 20 knee joints (10 with

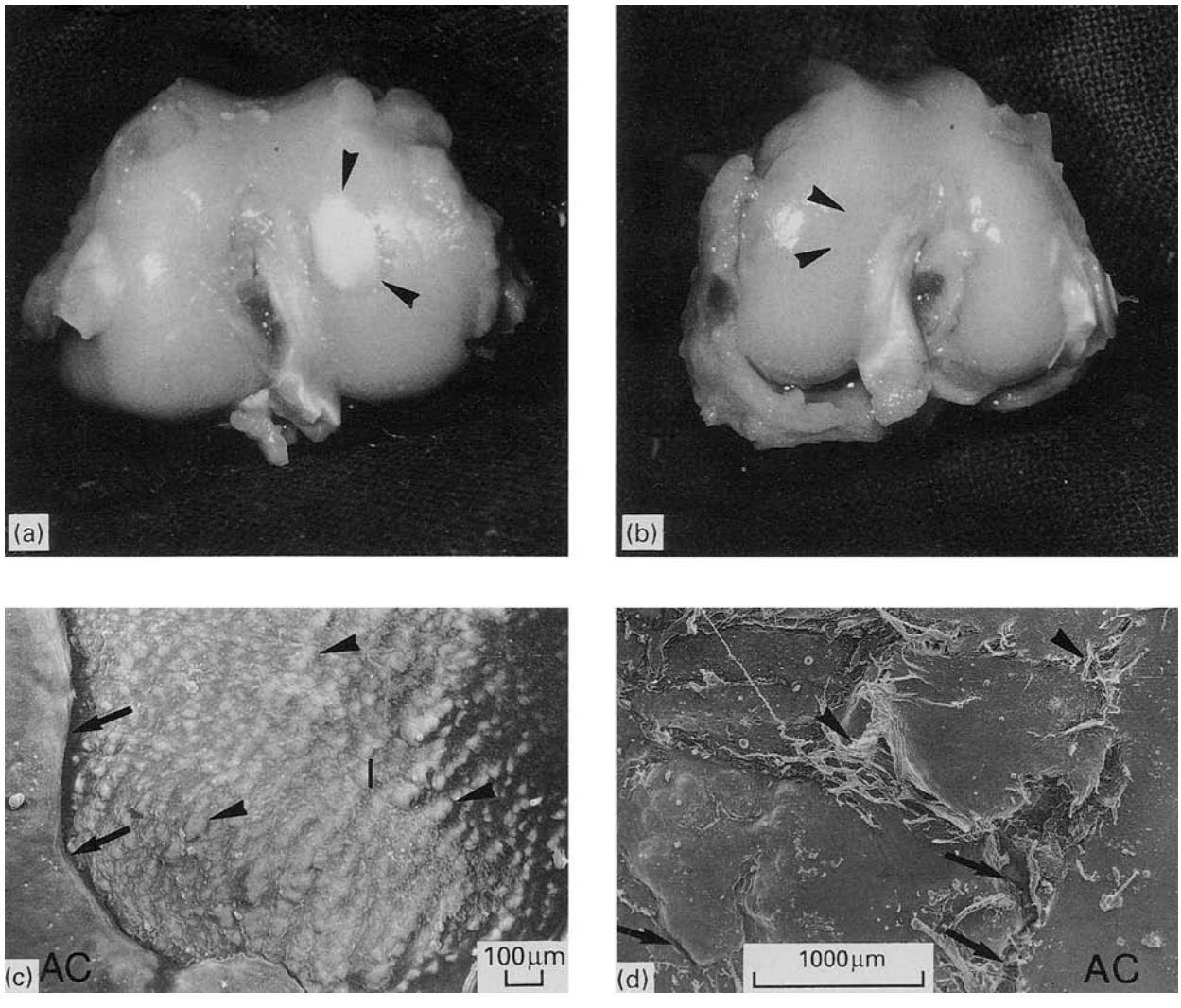


Figure 3 (a) Femoral condyles with e-PTFE implant after 14 months. The implant seems to be well integrated in the articular surface and covered partly by a thin translucent tissue layer (arrow-heads). (b) Femoral condyles in a control knee-joint (control defect). The articular surface is smooth. On the medial condyle (left) a slight shift in colour denotes the location of the defect (arrow-heads). (c) Survey scanning electron micrograph of an articular surface with an e-PTFE implant (I). The surrounding surface of the articular cartilage (AC) is smooth. The edge of the cartilage appears to partly overlap the implant (arrows). The implant surface has numerous oval-rounded elevations (up to about 100 μm in diameter) (some of which are indicated by arrow-heads) which gives the surface a nodular character. (d) Survey scanning electron micrograph of an articular surface in a control knee-joint (control defect). A part of surface (presumably related to the healing site of the created defect) has an uneven surface topography. The area is partly separated from the surrounding, smooth articular cartilage (AC) by crevices of varying dimensions (arrows). The circumference of a partly detached area of the surface has a fibrillated character (arrow-heads).

implants and 10 controls) in 10 rabbits killed after 14 months.

3.1. Macroscopic observations

No signs of swelling or hydrops were observed in any of the knee joints. The synovia were pale. The implants were found in place and mostly covered by a thin translucent tissue layer (Fig. 3a). In the control knees, the corresponding defect site varied from smooth to (in most cases) an irregular surface (Fig. 3b). In five of the animals osteophytes could be seen at the medial condyle in both implanted knee joints and controls. These animals did not differ from the other animals with regard to joint swelling or the tissue around the implants.

3.2. Scanning electron microscopy

The surfaces of the two e-PTFE implants examined with scanning electron microscopy had a nodular appearance (Fig. 3c). The fibrillar areas of the e-PTFE sheath were not visible, instead the surface appeared to be covered by an amorphous layer. The cartilage which bordered the implants had a smooth contour and was separated from the implant. Inflammatory cells were rarely detected on the surfaces of the implants. The exact location of the surgically created

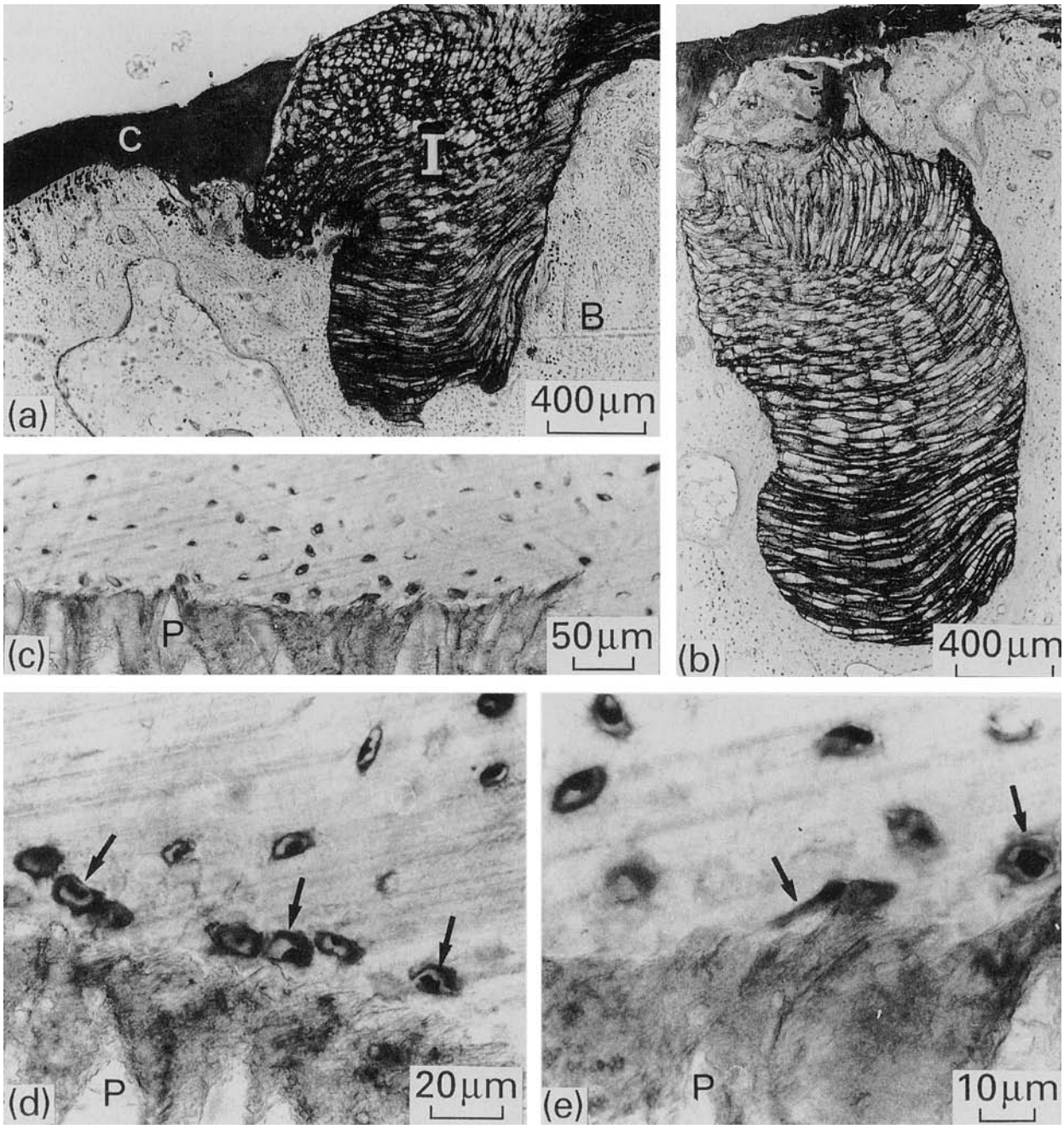


Figure 4 Micrographs of the medial part of the joint. (a) Survey (LM) of the implant (I) in level with the articular cartilage (C) and the subchondral bone (B). BM = bone marrow. (b) The e-PTFE implant is surrounded by compact bone. Part of the articular surface consists of e-PTFE (arrow). (c) The surface of an implant (I) is observed in contact with the bone. Several osteocytes are located close to the implant surface. P = pore within the implant. (d, e) Details of previous area, showing osteocytes (some of which are indicated by arrows) immediately adjacent to the surface of the porous e-PTFE implant. P = pore.

defect in the control joints was difficult to discern. However, in both control knee-joints examined, a central part of the condylar articular surface was markedly irregular (Fig. 3d). Areas of the surface were partly detached from the surrounding cartilage as indicated by spaces and fibrillar appearance.

3.3. Light microscopy

Light microscopic observations on ground sections showed that the e-PTFE implants occupied the surgically created defects in the articular surfaces. The cartilage adjacent to the implant had a fibrocartilage-like appearance, often with clusters of chondrocytes

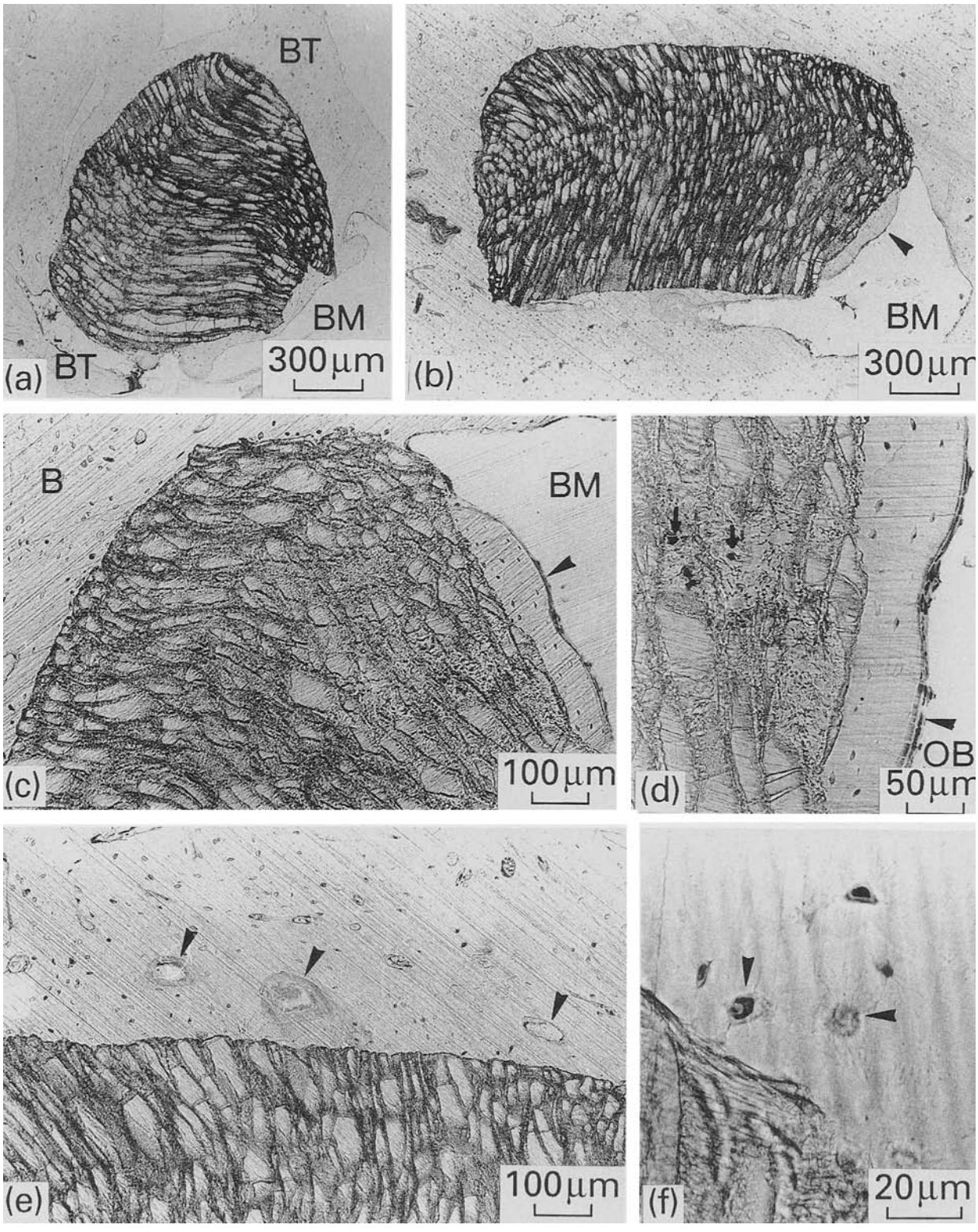


Figure 5 Light micrographs of the lateral area of the joint. (a) Bone trabeculae (BT) surround the e-PTFE. BM = bone marrow. (b) The implant surrounded by condensed bone. A bone marrow space (BM) is partly separated from the implant surface by an "island" of bone (arrow-head). (c) Detail of the border between the bone marrow space (BM) and the implant. The "island" of bone (arrow-head) is located on the surface of the implant, without an intervening connective tissue. B = bone. (d) Darkly stained cells (arrow-head) are observed within the pores of the e-PTFE. The "island" of bone (B) is lined by a thin layer of osteoblasts (OB). (e) The implant is surrounded by condensed bone which contains several blood vessels (arrow-heads). The bone is in contact with the implant surface. No tissue can be detected inside the porous e-PTFE. (f) Osteocytes (some of which are indicated by arrow-heads) are located close to the implant surface.

(Fig. 4a). In several sections, a space with varying width was found to separate the implant from the cartilage. The joint cartilage on the tibial plateau had a normal histological appearance also in the regions facing the e-PTFE implanted area of the femoral condyle.

A striking and consistent finding was the large amount of bone around the implants. This was evident in all planes of section (medially and laterally). In the subchondral area the bone had formed a collar around the implants (Fig. 4b). In general, the bone was in direct contact with the e-PTFE surface (Fig. 4c). Osteocytes were often located immediately adjacent to the implant surface (Fig. 4d, e). Sections which were obtained from the mid and lateral condylar area revealed a similar morphology. It was a general impression that a merging and condensation of bone trabeculae had occurred around the implants (Fig. 5a, b), with osteocytes adjacent to the implant (Fig. 5c). In areas where bone marrow was located close to the implant surface, bone often separated the implant from the marrow space (Fig. 5d, e). These "islands" of bone, which in the sections did not have any contact with remaining bone, were in direct contact with the implant surface (Fig. 5f).

In general, it was hard to evaluate the presence of cells and tissue within the porous structure of the implant. Infrequently, cells could be detected deep inside the implant (Fig. 5d). However, in general there was little tissue present within the implant (Fig. 5e, f). The synovial specimens showed a normal histological picture with few inflammatory or multinuclear giant cells. Examination in polarized light did not reveal any signs of wear particles in the tissues.

3.4. Morphometry

The results of the morphometric evaluation of the e-PTFE-tissue contact are summarized in Table I. In section A (subchondral area) 61.0% was in contact with bone, 6.1% with bone marrow and 4.1% with loose connective tissue (e.g. in connection with blood vessels). Dense connective tissue, was seen in contact with the implant surface in 28.8% of the total implant length in section A. Inflammatory tissue in contact with the implant could not be seen in these sections. In section B (midcondylar area) 83.1% of the total length of the implant surface was in contact with the bone, 6.7% with bone marrow and 4.0% with loose connective tissue. In section C (lateral condylar area) 75.1% of the total implant surface length was in contact with bone, 13.6% with bone marrow and 3.4% with loose connective tissue. Dense connective tissue was seen to be in contact with 6.5% of the total length of the implant surface. The summarized measured lengths (mean values in per cent) of the e-PTFE implant in contact with different tissues in the medial femoral condyles (sections A + B + C) showed that 73.1% was in contact with bone, 8.8% with bone marrow and 3.8% with loose connective tissue.

4. Discussion and conclusions

Repair of damaged articular cartilage has been tried using many different methods and materials. Resurfacing arthroplasties with various types of biological or synthetic materials have been tried (4, 22–24). E-PTFE is suggested to be a biologically and chemically inert material when used for tissue reconstruction [18, 25]. In the present study, cartilage was not formed over the surface of the implant. This observation is in contradiction to previous findings using e-PTFE and shorter follow-up periods [9]. However, all the implants had stayed in place and filled the defects in the joint articular surfaces with fairly close contact between surrounding cartilage and the e-PTFE membrane. The tissue adjacent to the implant consisted of apparently newly regenerated fibrocartilage. The degenerative changes that has been described for osteoperiosteal and perichondral grafts after 6–12 months [3, 26] were not found. Implant encapsulation by fibrous tissue, tumour formation or the spreading of wear products, which has been reported for other materials, e.g. silicone [27], were not observed in the present study 14 months after implantation. The surface of the e-PTFE (facing the joint cavity) had few adherent or incorporated cells as judged by LM and SEM. The surface had a nodular but otherwise smooth character without the presence of visible pores. It cannot be excluded that, for instance, a fragmentation of the surface of the e-PTFE with a generation of material products could have occurred at an earlier time.

The presence of fibroblast-like cells in the membrane, reported earlier in a short-term study [9] could not be observed. In the present study there were few cells detected inside the porous material. At present we have no explanation for this observation. In the present study no signs of infection in association with the implants was observed.

The porous structure of the implant and the lack of cells inside these pores may be a disadvantage from a clinical point of view: spaces which are secluded from the tissue exterior might be areas which are prone to

TABLE I Morphometric evaluation of the length of the e-PTFE implant in contact with different tissues at the three section locations (A, B and C) in the femur condyle (mean values in % ± SEM; n = 8)

Section	Mineralized bone	Bone marrow	Loose connective tissue	Dense connective tissue	Inflamed connective tissue
A	61.0 ± 14.4	6.1 ± 5.3	4.1 ± 2.7	28.8 ± 16.7	0.0 ± 0
B	83.1 ± 4.9	6.7 ± 3.6	4.0 ± 1.3	6.2 ± 4.3	0.0 ± 0
C	75.1 ± 4.5	13.6 ± 3.8	3.4 ± 2.8	6.5 ± 2.9	1.4 ± 1.4
A + B + C	73.1 ± 6.5	8.8 ± 2.4	3.8 ± 0.2	13.6 ± 7.5	0.5 ± 0.5

infection. Apart from the invasive properties of the microorganisms, factors which could be of importance for development of infection include the surface characteristics of the surface in the interior of the material, the adsorbed proteins and the content of a possible exudate within these spaces. Further, the availability of the local defence systems (including the degree of vascularity and the recruitment of inflammatory cells) may be quite different within a porous implant from that at the surface of a solid implant.

Despite the observation that the membranes were not covered by articular cartilage we did not find any structural damage to the opposite articular cartilage on the tibial plateau. Further studies are required in order to determine if the structure and surface characteristics of the membrane could make it suitable as an artificial joint surface in smaller non-weight-bearing joints.

The interaction between bone and different implants has been extensively studied, especially between bone and titanium. Titanium has been shown to heal in close contact with bone (often called osseointegration) [28–31]. In dental surgery for treating edentulism, the Brånemark method for osseointegration of titanium implants has been utilized for more than 20 years [28]. A prerequisite when using this method is a sufficient quantity of bone. One important factor for bone regeneration is the prevention of soft tissue ingrowth, which may otherwise disturb osteogenesis in a bone defect. The use of e-PTFE membranes as physical barriers and seals in order to prevent the influx of non-osteogenic cells has been shown to promote the healing process towards bone generation [32].

A conspicuous morphological feature in the present study was the large amount of bone around and in contact with the surface of the e-PTFE implant. The degree of bone–implant contact was higher than the values reported for threaded titanium implants (about 40% bone–implant contact in the rabbit trabecular bone (knee-joint) 6 months after surgery [33]). The values observed in the present study are in parity with data presented for threaded titanium implants inserted in the tibial metaphysis [34]. The latter study showed an average of 85% contact between bone and titanium implant (in the four best consecutive threads) 1 year after surgery [34].

A high degree of contact between bone and the e-PTFE implant, without any signs of intervening fibrous capsule, and the presence of osteocytes in close contact with the membrane surface was observed. This could imply that e-PTFE may be an interesting material for various reconstructive surgical procedures within the fields of orthopaedics and hand surgery. One important factor for the long-term results may be the initial fixation of the material [31]. This was achieved in this study by inserting the membrane via drilled channels through the femoral condyle and firmly securing the membrane extra-articularly on the dorsolateral side of the condyle. The role of the initial firm fixation for the incorporation of e-PTFE is currently being examined.

Also from a theoretical perspective, the finding of an integration of e-PTFE in bone after 14 months, is highly interesting. Previously, a series of poly (ethyleneoxide) poly (butylene terephtalate) (PEO/PBT) copolymers have been found, depending on their composition, to possess properties which promote an intimate bone–implant contact [35]. Several properties which are attributed to e-PTFE, for instance the hydrophobic surface and the porous structure, are amenable to systematic modifications. The role of these surface properties in the contact between bone and the polymer remains to be established. It is too early to speculate on the possible mechanisms for the present findings. Based on the present LM observations it cannot be excluded that a thin layer of non-calcified tissue intervenes between the PTFE surface and the mineralized bone. Therefore, current projects are concentrated on obtaining intact thin and ultra-thin sections of the intact e-PTFE-bone interface in order to unravel the fine structure of the polymer–bone interface. Most likely, due to preliminary difficulties with calcified e-PTFE specimens, part of this work will have to be done on decalcified specimens, and also with additional analytical tools.

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