JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 9 (1998) 509–515

Bone remodelling in the pores and around load bearing transchondral isoelastic porous-coated glassy carbon implants: Experimental study in rabbits

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Cylinders of porous-coated glassy carbon were implanted into drill holes made through the articular surface of the medial condyle of both tibiae of ten rabbits for six and 12 weeks. Bone ingrowth and remodelling was examined by radiographic, histologic, oxytetracycline-fluorescence and microradiographic methods. Bone ingrowth into pores and load bearing implants was seen by all examination methods. Bone ingrowth occurred earlier when the pores were facing cancellous bone than cortical bone. Appositional bone formation occurred on the trabeculae a few millimetres from the interface during the early phase of remodelling at six weeks. At 12 weeks resorptive remodelling had occurred both in the surroundings and in those pores that face cancellous bone, whereas the amount of bone still increased in the pores facing cortical bone. In its porous-coated form glassy carbon functions well as a frame for ingrowing bone and it shows good osteoconductivity. Its mechanical properties are suitable for functioning as a structural bone substitute in places where the loads are mainly compressive. The difference between findings at six and 12 weeks indicated physiologic stress distribution and the adverse effects of stiff materials on bone remodelling were avoided by using this isoelastic material.

1. Introduction

Bone grafting is used to reconstruct or replace skeletal defects, to augment fracture repair, to strengthen arthrodeses and fill defects after the treatment of bone tumours. Autologous bone has been the standard of care. The morbidity associated with augenous bone graft harvest [1, 2] and public awareness regarding the transmission of a live virus [3] through use of allografts, have been the impetus for research into a variety of materials that could be used instead of standard materials for bone grafting.

In addition to osteoinductivity and osteoconductivity of bone grafts the design and the mechanical properties of porous material for bone ingrowth must consider the need for stress transfer from the prosthetic device to the surrounding bone. Reconstructive challenges, particularly those involving joints, have been addressed by synthetic alternatives. Metals and plastics have been used with great success in replacing articular surfaces and, to a lesser degree, segmental bone loss. The mechanical properties, especially modulus of elasticity of the implant may play a significant role in the resulting remodelling process. One of the major problems in orthopaedic surgery has been the mismatch between bone and implant stiffnesses. A graft should be able to support a load, when necessary. It is this task of bone substitute, where the subject of this study, glassy carbon, is expected to contribute.

The *glassy carbons*, the objects of this study, are formed in an oxygen-free environment by controlled heating of a solid, preformed polymeric body to drive off volatile constituents, leaving a glassy carbon residue. Our precursor in fabricating amorphous carbon has been phenol-formaldehyde. The heating rate must be low enough to allow the volatile by-products to diffuse to the surface and escape and thus avoid the formation of bubbles. With increasing specimen

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thickness the carbonization time increases, as also does the risk of destruction of the formed carbon.

We were initially attracted to glassy carbon because of its record of biocompatibility [4–6]; as well as to several of its mechanical parameters, e.g. modulus of elasticity, being similar to that of cortical bone. When we started the general opinion was that it was impossible to fabricate artifacts out of glassy carbon thicker than 3 mm [7] or 7 mm [8] without fissuring during the carbonization process. The potential to fabricate larger devices, without losing the characteristic properties of glassy carbon materials, by using the modification of Rautavuori and Törmälä [9] was appealing. Modification of the preparation made it possible to prepare bulky, mechanically strong, microporous glassy carbon bodies with maximal thicknesses of up to 20 mm. It is this glassy carbon, prepared by the modification described by Rautavuori and Törmälä [9] that is the object of the present study.

A 12 week period was necessary for maximum tissue development into glassy carbon implants in animal model [10, 11]; this has also been shown by using Co–Cr–Mo alloy [12]. Bone resorption caused by stress protection has previously been reported to occur with metallic porous implants fixed by bone ingrowth [13, 14].

To study the effects of dynamic loading to biologic fixation of porous-coated glassy carbon implants an experimental study was designed. Our hypothesis was that a low modulus allows the near normal remodelling of bone in the pores of the implant in a behaviour not provided for in more rigid porous metals [13, 14].

2. Experimental procedure

2.1. Implants

The glassy carbon implants were prepared as cylindrical pellets, 4 mm long and 4 mm in diameter. Each implant had a microporous core of glassy carbon (10–100 nm) prepared by a previously published method [9]. These cores were coated with porous glassy carbon; except for one end, which remained smooth. The coat was 1 mm thick, its pore volume was 55% and the average pore size 200–300 μm.

2.2. Experimental animals and surgical procedure

Ten rabbits, weighing 1900–3040 g, were operated on under intravenous Nembutal®–anaesthesia. Both knee joints of each rabbits were exposed through the medial parapatellar approach. The medial meniscus was removed from each joint. A drill hole, 4 mm in diameter and 4 mm deep, was made through the proximal medial articular surface of the tibia (Fig. 1). The implants were inserted into the holes with press fit. The smooth end of the implant was adjusted to the level of the articular surface by the exact depth of the drill hole. Procain Penicillin(100 000 IU) was administered intramuscularly in order to avoid septic complications. Post-operatively, the rabbits were allowed to move freely in the cages. The rabbits were sacrificed six and 12 weeks post-operatively, five in each group.

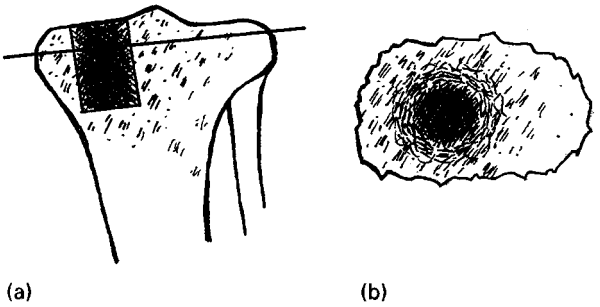

Figure 1 A sketch illustrating (a) the location of the implant and (b) the direction of sectioning.

2.3. Radiography

After sacrifice, bones were cut transversely immediately beneath the implants. Radiographs were taken of each specimen, the beams going parallel with the axis of the implant. A three-phase roentgen generator and a special microfocus tube (0.14×0.14 mm, 3 kW) were used at 50 kV, 32 mA and 16 mAs.

2.4. Histology

The specimens were dehydrated by bathing successively in a graded series of 50% to absolute alcohol which was replaced by xylene and later embedded successively in methylmethacrylate. For histological analysis transversal sections of 5 μm were made (Fig. 1) using a Reichert–Jung microtome, and stained by the modified Masson–Goldner method [15]. A Leitz light microscope was used for examination of the slides.

2.5. Microradiography and oxytetracycline (OTC)-fluorescence

For OTC-fluorescence studies the rabbits were given an intramuscular injection of oxytetracycline (Terramycine®, 50 mg kg^{-1} of body weight) 48 h before sacrifice. For OTC-fluorescence study, the sections were examined under ultraviolet light for new bone observation [16]. Transversal sections (Fig. 1) of 80 μm were used for OTC-fluorescence and microradiographic examination.

3. Results

3.1. Macroscopical

One implant was broken and the loose part had disintegrated into the joint. The synovium in that case was black, stained by the particles. Tissue that resembled cartilage tended to grow from the periphery over the impant. In one case after 12 weeks of implantation, the implant was fully coated by this tissue. Both six and 12 weeks after implantation loss of cartilage was observed in the opposite medial femoral condyle.

3.2. Radiography

The implants were translucent in the radiographs. At six weeks a radiopaque ring surrounded the microporous core of the implant representing the outer parts of porous coating and the immediate surroundings of the

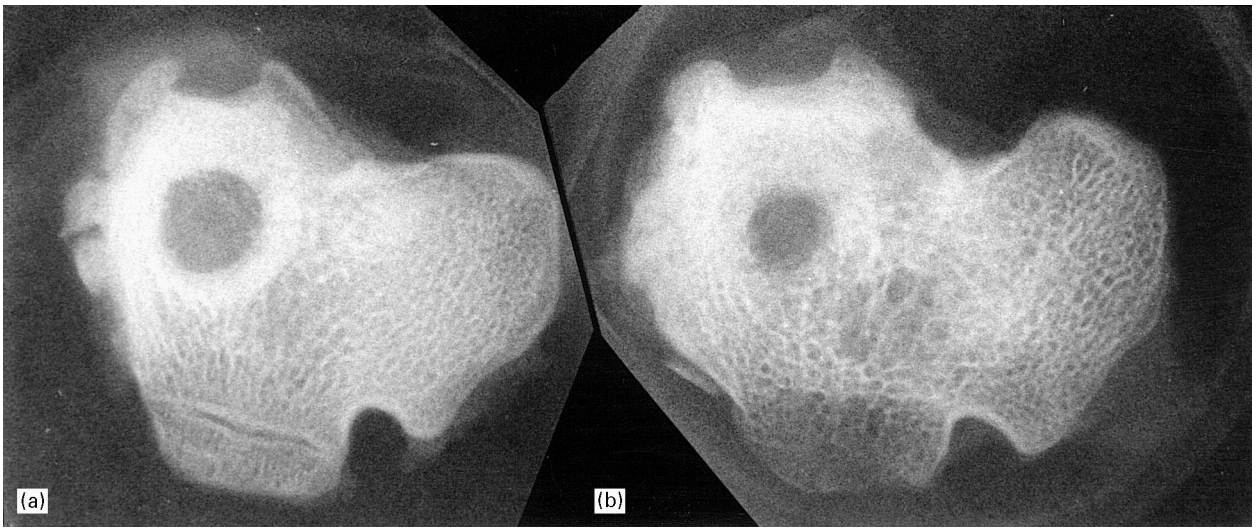

Figure 2 Radiography of the specimen: (a) six weeks–Radiopaque circle around the implant indicating appositional bone formation on the trabeculae of the surrounding bone; and (b) 12 weeks–resorptive remodelling has occurred around the implant (the porous coating is not radiolucent any more; its radiopacity in the porous area and interface resembles the radiopacity of the remodelled surrounding bone).

implant (Fig. 2a). At 12 weeks the radiopacity surrounding the implant was not as apparent. The density of the coating area resembled the density of the surrounding bone (Fig. 2b). No radiolucent areas were seen.

3.3. Histology

In sections, glassy carbon appeared as a circle. The middle of the implant crumbled during the cutting procedure. The glassy carbon surface was in contact with cancellous bone, cartilage and marrow, depending on the level of the sectioning. In places where the implant was in contact with bone, there was bone in the pores. In places where contact was with cartilage, there was no bone ingrowth; whereas in contact with medullary tissue, in addition to medullary tissue in the pores, there was some bone in the pores. At 12 weeks the tissue in the pores and in the vicinity of the implant had remodelled: the trabeculae were continuing into pores and in places where the implant was in contact with marrow tissue the same tissue was found in the pores, too (Fig. 3). Some macrophages were seen, but no giant cells. At 12 weeks there was intimate contact between the bone and implant in those sites where after remodelling the surrounding trabeculae continued into the pores and bone occupied a considerable part of the total area of the ingrown tissue (Figs 3 and 4). There were many lines of rounded osteoblasts and thick osteoid seams as well in the pores as in the surroundings at six weeks (Fig. 5a). In the surrounding bone at six weeks the osteoblastic lines on the trabeculae were more pronounced and more numerous than at twelve weeks. The osteoid seam between the osteoblasts and bone was thicker at six weeks than at 12 weeks. At 12 weeks remodelling seemed to be complete in the surrounding of the implant with only isolated osteid borders on lamellar cancellous trabeculae (Fig. 5b). The osteoblasts on the trabeculae were flat and fusiform with thin osteoid seams (Fig. 5b).

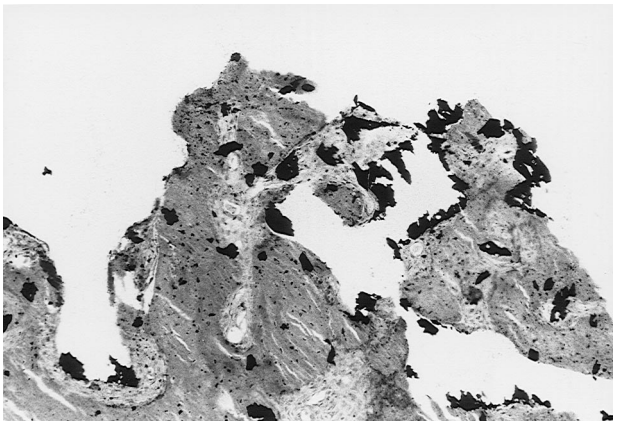

Figure 4 Histology at 12 weeks: striped dark grey area representing bone occupies most of the area of tissue spicule grown into the pore of the implant. Black spots are remnants of the implant that has crumbled during the cutting procedure. Intimate contact between the bone and implant can be seen on the upper part of the spicule. Magnification $\times 40$.

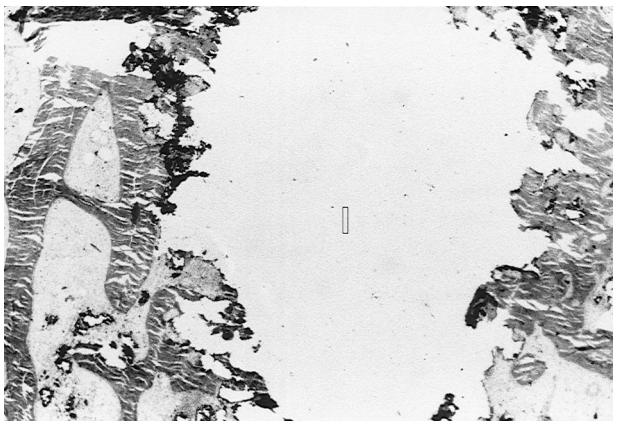

Figure 3 Histology at 12 weeks: the large white area in the middle represents the implant that has crumbled during the cutting procedure. The remodelled surrounding tissue continues into the pores of the implant as spicules. On the right the spicules are totally occupied by mature bone (striped, dark areas). On the bottom of the right-hand side and on the left, light marrow containing spicules can be seen. Magnification $\times 10$.

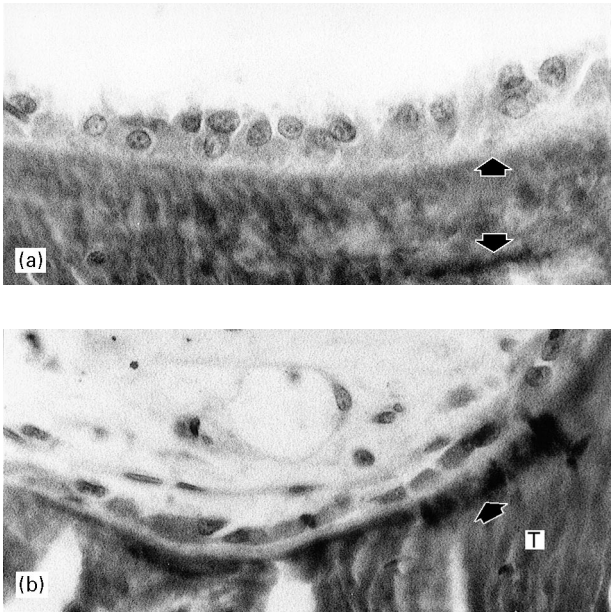

Figure 5 Histology at (a) six weeks [a line of active rounded osteoblasts and a thick osteoid seam (between arrows) on the trabeculae surrounding the implant] and (b) at 12 weeks [Resting-type oval osteoblasts and a narrow osteoid seam (arrow, T = trabecular bone)]. Magnification ×10.

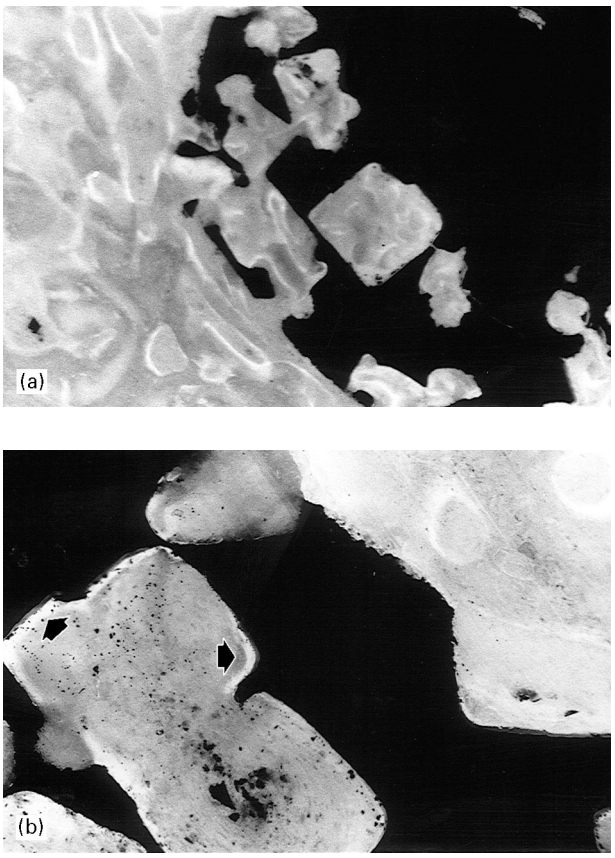

Figure 6 OTC-fluorescence at (a) six weeks (active bright labelling on the trabeculae outside the implant and in the pores–magnification ×10), and (b) at 12 weeks [bright labelling (arrows) in the periphery of the pore–magnification ×40].

3.4. Fluorescence microscopy

At six weeks the fluorescence was noticed both around the implant and in the pores (Fig. 6a). At 12 weeks the fluorescence was not so profound in the surroundings of the implant. Fluorescence was still seen in the pores in the periphery and often in contact with the wall of the pore (Fig. 6b).

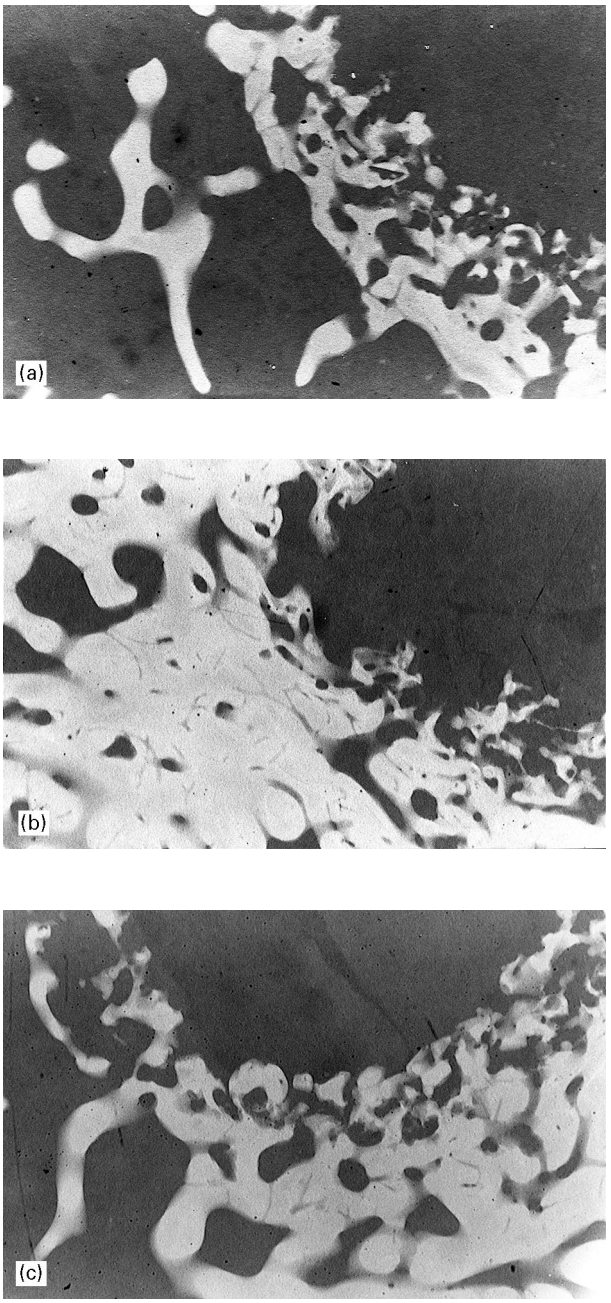

Figure 7 Microradiography: (a) implant (upper right) in cancellous bone at six weeks showing intensive bone formation in the pores and interface; (b) implant (upper right) in cortical osteonal bone at six weeks–there is less bone in the pores than in (a); and (c) Implant at 12 weeks in site where it is in contact both with cancellous (left) and cortical (right) bone (to the right the bone spicules in the pores have modelled into osteonal bone; to the left the circle of lamellar bone at the interface seen in (a) has disappeared and the bone spicules in the pores have remodelled into a cancellous structure resembling the surrounding cancellous bed). Magnification ×10.

3.5. Microradiography

At six weeks a circle of mature lamellar bone was seen surrounding the implant in the cancellous bed (Fig. 7a) and in the pores there was newly formed less radiopaque bone both in the implants in the cancellous (Fig. 7a) and cortical (Fig. 7b) beds. There

was a slight difference between samples at six and 12 weeks and between ingrowth of cortical osteonal and trabecular bone. At 12 weeks the coating was more uniformly filled with radiopaque lamellar bone and its opacity was the same as in the surroundings of the implant (Fig. 7c). The trabeculae of the surrounding cancellous bone continued into the pores and in places where it was in contact with osteonal bone the ingrown bone resembled osteonal bone (Fig. 7c). In places where the implant was in contact with radiolucent tissue there was no bone ingrowth into pores. At six weeks in places where the pores were facing cancellous bone (Fig. 7a) the amount of bone in the pores was greater than when the pores were in contact with cortical bone (Fig. 7b). At 12 weeks the amount of cortical bone had increased and the amount of cancellous bone remained at the same level or decreased compared with the amount noticed at six weeks (Fig. 7c). Lamellar circular structure surrounding the implant at six weeks (Fig. 7a) had disappeared at 12 weeks and the tissue surrounding the implant had remodelled and continued into the pores (Fig. 7c).

4. Discussion

The modulus of elasticity of bone is given as 10–20 GPa for compact bone [17]. The elastic modulus of individual trabeculae in different studies varies from 1 to 20 GPa [18]. Most publications consider an average value for both elasticity of 17.6 GPa [19].

Individual trabeculae and cortical bone can be mechanically viewed as a single material of variable density [20, 21]. Ashman and Rho disagree: they expect the elastic modulus of individual trabeculae to exhibit a 25% lower value than cortical bone; a value of approximately 15 GPa [22]. The compressive strength of bone tissue is proportional to the square of the apparent density, and the compressive modulus is proportional to the cube of apparent density [23].

A difference should be seen between the individual trabeculae and the porous cancellous structure. Cortical bone is 30 times stronger than cancellous bone and the elastic modulus of cancellous bone is one-thirtieth of that of compact bone [24]. In other words, the elastic modulus of cancellous bone is an order of magnitude < 1 GPa.

In comparison, modulus of metacrylate bone cement is 2 GPa, and the most commonly used metals in prosthetics, Co-alloy and Ti-alloy have moduli of 200 MPa and 100 GPa, respectively [17]. Porous metallic coatings have lower moduli than the non-porous substrate. Values ranging between 50 to 150 GPa have been suggested for Co-alloy porous systems [25]. For the material we used, glassy carbon prepared by the modification by Rautavuori and Törmälä [9], elastic moduli have been given as 40 GPa in compression and 17 GPa in bending [26].

When properly designed and implanted porous-coated implants are well fitted to bleeding bone, an initial fracture healing response occurs that results in bone ingrowth at approximately six weeks post-operatively [27]. This is followed by a remodelling phase, which, according to our hypothesis based on Wolff's law [20], is dependent on the elasticity of the implant. Any material to be used as a substitute for cancellous bone must allow elastic deformation and load distribution without any stress protection or stress concentrations.

The present study confirms the occurrence of bony ingrowth in a porous-coated glassy carbon implant under conditions of loading. The loading was ensured by the level of the implant surface, which was planned to be on the level of the surface of the cartilage. The elasticity of the surrounding cartilage assured the real loading of the implant, which was proved by local cartilage damage noticed regularly on the opposite femoral condyle at harvesting.

It is reasonable to discriminate between the early effects of an implant material on the processes during wound healing, i.e. bone healing, which exert an influence on the development of immature tissue, and the late effects, which modify the turnover of a more mature tissue, in the case of bone, the remodelling. Bone remodelling occurs throughout life by replacement of old bone with new bone. Also the ingrown bone in the porous coating will later be remodelled.

Increased bone density around the implants noticed at six weeks in radiographs (Fig. 2a) reflects enhanced appositional bone formation occurring on the trabeculae a few millimetres from the interface (Figs 2a, 5a and 6a) and the repair process, where surgical damage mimics an internal cancellous graft and appositional bone formation occurs without any preceding resorption [28].

The histologic, oxytetracycline-fluorescence, microradiographic and radiologic findings indicated physiologic stress distribution at 12 weeks. There has been a stabilization of the union of original cancellous and cortical bone, newly formed bone and the implant (Figs 2b, 3 and 7c). This equilibrium is in accordance with Wolff's law [20]. The bone in the pores in the original cancellous area had remodelled into cancellous bone and the bone in the original osteonal cortical area had remodelled into cortical bone (Fig. 7c). The findings did not indicate any stress concentration or stress shielding effects of the implant.

The difference in the rate of bone formation in the pores in cancellous and cortical bone can be explained by the difference of the vascularity [29] and the presence of cells with osteogenic potential. Differences in osteogenic potency of tissue related to the anatomical site have been demonstrated [30]. The difference between the rate of bone ingrowth into implant pores in cancellous and cortical beds resembles the difference between the duration of the fracture repair of cancellous and cortical bone as well as the incorporation of cancellous and cortical autografts, respectively.

In contrast to our study using an isoelastic implant, extensive bone remodelling was still under way at the 12-week post-implantation time period adjacent to and within a high modulus implant of Co–Cr–Mo alloy [31]. Garetto *et al.* [32] showed that sustained elevation of bone turnover adjacent to titanium implants was seen in four different animal species. The physical mechanism for stress concentration at the

interface of an implant within dynamically loaded bone was supposed to be due to the mismatch in the modulus of elasticity between titanium and bone [32]. Because of the large elastic modulus differences between bone (17 GPa, [19]) and metal (100–200 GPa, [17]), large stress concentrations can occur in the bone surrounding the implants. Highly stiff metal implants bonded by bony ingrowth can cause stress shielding of bone resulting in bone resorption due to disuse atrophy [13, 14]. In clinical post-mortem material Bloebaum *et al.* [33] found only 12% bone ingrowth in porous coatings of successful acetabular titanium implants. Earlier retrieval studies using clinical material have revealed that extensive ingrowth of bone does not occur regardless of whatever coating system or metal is used [34–36]. The relatively modest amount of bone in the pores can be explained by mechanical factors; the resorptive remodelling in the pores due to stress shielding. No more bone in the pores is needed in that model to redistribute the loads.

The results of our study show that new bone is strongly influenced by the osteogenic capacity of the surrounding tissues in the repair phase and by the mechanical factors in the remodelling phase. The results indicate physiologic stress distribution in the surroundings of the implant, at the interface and even reaching the ingrown bone in the pores. The maintenance of anchorage of clinical porous-coated implants has been a concern. Our study confirms the hypothesis: isoelasticity could be the solution.

According to Adams *et al.* [37], Kaae [38] has compared the properties, showing that, for a typical glassy carbon in three-point bending, the fracture stress is 225 $MN\,m^{-2}$, the strain energy to fracture 10 MPa, and the strain to fracture 1%. Strain to fracture illustrates the total deformation that exists at the time of fracture. All carbon-base materials are brittle in that they show no significant permanent or plastic deformation prior to fracture. Though carbon seems to be ductile compared to aluminium oxide, the strain to fracture of which is only 0.1%.

The strain energy to fracture of glassy carbon is particularly low [38]. Stanitski and Mooney [39] admitted after their animal study that glassy carbon is too brittle to withstand functional loading in skeletal prostheses. We do not recommend its use in places where it will be subjected to tensile or bending stresses. The mechanical properties of glassy carbon are sufficient in locations where the stresses are mainly compressive. The maximum shear strength between bone and porous-coated glassy carbon (4.6 $MPa\,m^{-2}$) reached at six weeks is comparable to that of acrylic cement using conventional cementing techniques [40]. The limiting factor of the shear strength was the strength of the porous coating itself [40].

5. Conclusions

Glassy carbon can be used as a structural bone substitute and its elastic properties favour physiologic stress distribution. Glassy carbon lacks an inherent problem with metals and oxide ceramics in orthopaedics, the extreme stiffness difference, i.e. modulus mismatch, between the implant and its cancellous bone bed. It provides better structural support than bone grafts and bioactive ceramics. A severe disadvantage is the fragility of the carbon-porous coating. To improve osteoconductivity and to prevent abrasion of brittle porous-coating, other surface configurations and combining with osteoinductive factors or biodegradable materials should be considered.

Acknowledgements

This investigation was granted by The Medical Research Fund of Tampere University Hospital and the Academy of Finland.

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Received 4 October 1997
and accepted 26 January 1998