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# Bone growth into glassy carbon implants

## A rabbit experiment

This study was designed to evaluate the growth of bone into porous glassy carbon cylinders. Porous carbon cylinders were implanted in 30 rabbits intra-articularly in the metaphysis of the femur opposite from the patella. The rabbits were sacrificed up to 24 weeks after the operation. The bone samples were examined by histologic, fluorochrome and microradiographic methods. The amount of bone ingrowth was measured histomorphometrically.

Fluorochrome uptake was seen in the implant pores, which indicated new bone growth originating from surrounding bone. After 3 weeks, microradiographs revealed new bone formation in the pores and with time the bone spicules became more dense. The amount of bone tissue in the pores grew and reached a maximum at 12 weeks, when 45 per cent of the total pore volume was incorporated with bone tissue. No adverse tissue responses were observed.

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Non-porous glassy carbon obtained by pyrolyzing network-forming polymers in an inert atmosphere is superior to most other materials for implant application in living tissue according to Jenkins & Grigson (1979); various forms of carbon have already been used for prosthetic devices in humans (Bokros et al. 1976). In addition to its biocompatibility, non-porous glassy carbon has several other advantages such as chemical inertness, mechanical strength, low gas permeability and high temperature stability (McKee 1973). Its most critical disadvantage is that it is impossible because of fissuring, to make non-porous artifacts thicker than 3 mm; fissuring occurs during the fabrication process at temperatures where the greatest evolution of gaseous products is observed (Jenkins & Kawamura 1976). This severely limits the application of glassy carbon in orthopaedic surgery where bulky, strong implants are needed.

A method which makes it possible to prepare up to 20-mm-wide bulky, mechanically strong, microporous glassy carbon bodies which retain the typical mechanical properties of glassy carbon materials was published previously (Rautavuori & Törmälä 1979). This new material has potential application for implants in places where high absolute mechanical strength is required, e.g. bone substitutes and joint endoprostheses. Because the pore size (10–100 nm) is too small for ingrowth of bone tissue, the glassy carbon implants in this study were coated with a porous layer (pore size 200–300 µm) of the same material to allow bone ingrowth.

## Material and methods

### Implants

The glassy carbon implants were prepared at the Institute of Materials Science, Tampere University of Technology. The implants were cylindrical pellets, 4.5 mm long and 4.5 mm in diameter. Each implant had a solid central microporous core of glassy carbon (Rautavuori & Törmälä 1979); the core was coated with porous glassy carbon. The coating was 1 mm thick, the pore volume 55 per cent, and the average pore size 200–300 µm.

### Surgical technique

Thirty rabbits of both sexes weighing 2.4 to 4.6 kg were used in this study. During the operations the animals were under intravenous Nembutal® anaesthesia. Both knee joints of each rabbit were exposed through a medial parapatellar approach. Drill holes, 4.5 mm in diameter, were made through the articular surface of the femur opposite from the patella.

The implants were press-fitted into the holes so that the smooth surface of the implant leveled with the articular surface. The wound was closed in two layers. To prevent infection 100,000 I.U. Procain Penicillin was administered intramuscularly. Postoperative activity was in no way restricted. The rabbits were sacrificed 1, 3, 6, 12, and 24 weeks postoperatively, six in each group.

## Histologic, microradiographic, and OTC-fluorescence technique

The bone samples were fixed in 70% ethanol. After fixation the bone specimens were dehydrated in alcohol-acetone and embedded in methyl methacrylate. Five-micron-thick sections were cut with a Reichert Bio-Cut microtome equipped with an HK knife (Jung AG, Heidelberg, West Germany).

The bone sections were stained with Masson-Goldner trichrome stain. Morphometrical analysis was performed using an MOP-03 computerized, semiautomatic morphometric system. The areas of the pores as well as the areas of the bone formed in the pores were measured and the bone-to-pore percentage ratio was calculated.

For oxytetracycline (OTC) fluorescence studies the rabbits were given an intramuscular injection of 50 mg of oxytetracycline (Terramycin®) per kilogram of body weight 2 days before sacrifice. The bone sections to be used in the OTC-fluorescence and microradiographic studies were cut to a thickness of 80 μm.

## Radiographic technique

For radiographic examination, the femur was cut transversely at the level of the distal diaphysis and all soft tissue was removed. Both anterior and lateral views were taken with a 3-phase roentgen generator and special microfocus tube (0.14 × 0.14 mm, 3 kW) at 50 kV, 32 mA and 16 mAs. Focus size allowed geometrical three-fold magnification. Fuji RxG film with ultrafine grain intensifying screens (Siemens Rubin Super) was used. A standard developing system of 90 s was employed.

# Results

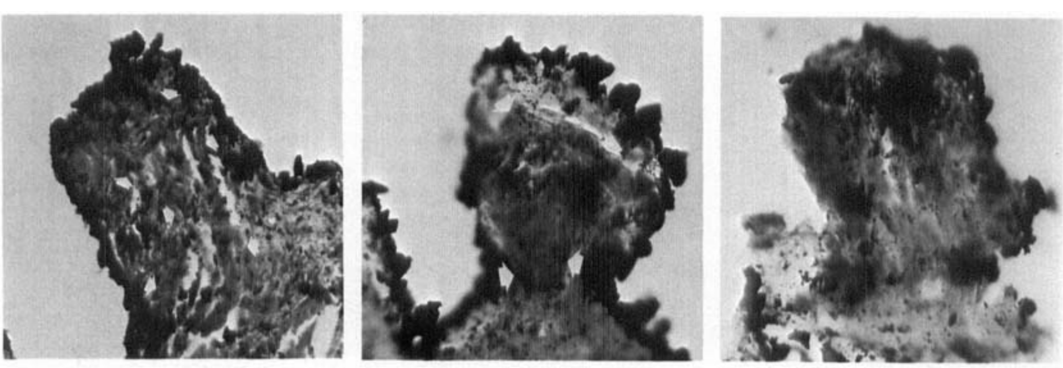
One medial dislocation of the patella, two subcutaneous and two intra-articular infections were found. Small carbon particles were seen in the synovial membranes. All implants were firmly attached.

## Radiographic findings

The glassy carbon material is radiolucent and so it was not clearly discerned. No translucent or sclerotic areas were noticed in the bone surrounding the implant.

## Histologic findings

One week postoperatively, there was haemorrhage around the implant, and connective tissue in the pores; no ossification was seen. After 3 weeks, there were osteoblasts, osteoid, and increasing amounts of mature bone in the pores. One and 3 weeks after implantation a fibrous tissue zone was found around the implant, but it was not seen after 6, 12 and 24 weeks. There was no foreign body reaction or inflammation (Figure 1).



A B C

Figure 1. Histology of the tissue in the pores of the implant after 3 weeks (A), 6 weeks (B) and 12 weeks (C). The carbon is only partly visible in the margin of the tissue because it crumbled during the cutting process. The white areas represent the implant and bone is visualized as grey blurred areas (arrows). The amount of bone increases with time and after 12 weeks the pore is completely filled with bone (×250)).

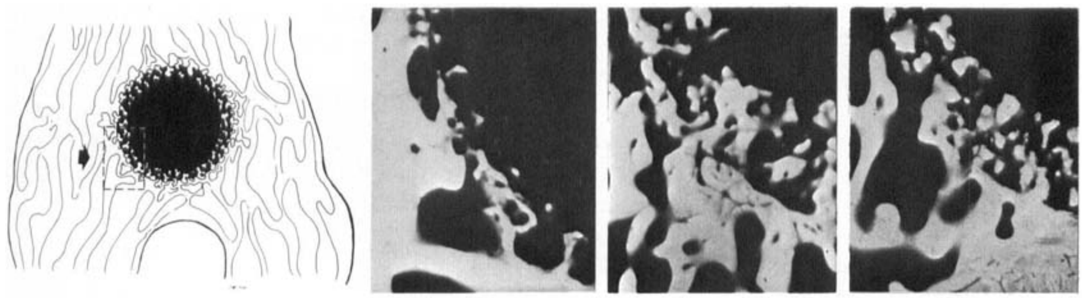



Figure 2. A. Transverse section from an implant outlining the location of the microradiographs after 3 weeks (B), 6 weeks (C) and 12 weeks (D). The carbon is not visible because of its radiolucency. Radiopositive bone is gradually formed in the pores of the implant (×40).

## Microradiographic findings

At 1 week the microradiographic examination revealed faint shadows just outside the implant. At 3 weeks faint shadows appeared in the pores in all of the samples. At 6 weeks the density of new bone had increased. At 12 and 24 weeks the bone density in the pores and in the surroundings of the implant was the same (Figure 2).

## OTC-fluorescence findings

At 1 week a thin scattered uptake was seen around the implant. At 3 weeks the uptake was observed also in the pores of the implant. At 6 weeks less labeled bone filled the pores and the interfacial bone-implant region than before. Later the uptake was minimal and some samples were without uptake. The labeling appeared first around the implant; later, when more recently mineralized bone appeared labeled deeper in the pores, the surrounding area was unlabeled (Figure 3).

Figure 3. A large tetracycline-stained deposit of new bone within a pore of the surface layer of the implant after 6 weeks (×250).

## Histomorphometry

Ossification in the pores reached a maximum of 45 per cent 12 weeks postoperatively (Figure 4).

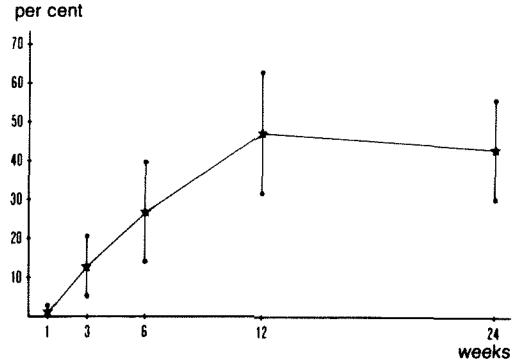



Figure 4. Percentage volume of bone in the pores of the implant versus implantation duration.

## Discussion

Bone grows into porous ceramic, metallic or polymeric biocompatible materials which have sufficient interconnecting pore size (Hulbert et al. 1970, Cameron et al. 1976, Spector et al. 1976). Hulbert and his associates showed that a pore size of 100 μm will allow bone ingrowth while a pore size of 150 μm will permit osteon formation. In a polymeric system, Klawitter et al. (1976) observed bone growth into porous high density polyethylene (HDPE) with a pore size as small as 40 μm.

We chose glassy carbon as the implant material because of its excellent biocompatibility (Benson 1972, Mooney et al. 1972). In addition, the elastic modulus of glassy carbon is very similar to that of bone, and thus stress will be transmitted across the junction between the implant and bone more uniformly than would occur with materials whose elastic moduli are much greater than that of bone (Grenoble & Kim 1973). Since manufacturing pieces larger than a few millimeters has been impossible, the use of glassy carbon has been almost totally limited to dental implants (Schnitman & Shulman 1980).

Three weeks after implantation, signs of bone in the pores of the implant were apparent with all our methods. The results indicate that the pores will be infiltrated first by fibrous tissue, which is then gradually replaced by bone. Histomorphometry revealed that the pores will not be completely filled with bone. Because glassy carbon is radiolucent, we could not evaluate the portion of bone in the pore volume microradiographically. The density did not increase from 12 to 24 weeks and in these groups the fluorescence was negligible, indicating that the ossification did not progress after 12 weeks.

The fibrous tissue zone, seen around the implant after 1 and 3 weeks, was replaced by bone in later groups, and the implant was in intimate contact with bone. The labeling appeared first around the implant and later, when more recently mineralized bone appeared labeled in deeper zones, the surrounding area was unlabeled. This centripetal direction of osteogenesis was also seen by histologic and microradiographic methods.

The implant trials with porous glassy carbon revealed no adverse tissue reactions induced by the material. The ingrowth of bone occurred at a rate of normal osseous repair. Other investigators have found this before in metals, ceramics and polymeric implants. The majority of the literature supports porous metal and porous ceramic systems. Carbon as well as polymeric materials appear to have potential from the standpoint of their lower elastic moduli (Morscher & Mathys 1975).

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