Acta orthop. scand. 53, 181–186, 1982

CHONDROGENESIS IN REPAIR OF ARTICULAR CARTILAGE DEFECTS BY FREE PERIOSTEAL GRAFTS IN RABBITS

JENS M. RUBAK, MIKKO POUSSA & VEJHO RITSILÄ

Department of Orthopaedic Surgery, Aarhus County Hospital, Aarhus, Denmark

The origin of the cartilaginous tissue in articular defects after periosteal grafting was studied histologically in 6-month-old rabbits. The grafts were taken from the tibia and transplanted to artificial defects in the femoral articular cartilage. An isolating Nucleopore filter®, hindering the penetration of cells, was placed between the graft and the cancellous bone, in order to trace the origin of the proliferating cells. The histological results revealed that the cartilage tissue which proliferated in the defect originated from the periosteal graft and not from the subchondral bone. The effect of the depth of the defect was studied by making a superficial and deep part in the defect. Cartilage tissue was found in both parts of the defect, though there was less in the more superficial defect.

Key words: articular cartilage repair; chondrogenesis; periosteal graft

Accepted 29.vi.81

The healing of cartilage defects has been described by many investigators (Redfern 1851, Shands 1931, Bennett & Bauer 1932, Carlson 1957, Mankin 1962, Campbell 1969, Sakakida et al. 1978). Superficial lacerations limited to the articular cartilage or to the calcified cartilage do not heal with regeneration of hyaline cartilage. The studies of Meachim & Roberts (1971), Salter et al. (1975) and Cheung et al. (1978) showed that small deep defects extending into the subchondral bone can heal by the ingrowth of cartilage tissue from the subchondral bone.

In a previous study (Rubak 1982) artificially created defects in the articular cartilage were covered with periosteal grafts and subsequently the defects showed proliferation of cartilaginous tissue.

The purpose of the present investigation, using Nucleopore filter isolating techniques, is to elucidate whether the cartilage tissue formed in the relatively large articular cartilage defects after free periosteal transplantation, originates from the periosteal graft or from the subchondral bone. Furthermore the effect of the depth of the defect on the chondrogenesis is evaluated.

MATERIAL AND METHODS

The experimental material comprised 112 knee joints in 56 rabbits weighing between 2500 and 3300 g. The operations were performed under circumstances described in a previous study (Rubak 1982). After transplantation of periosteum from the tibia to the articular cartilage defect, which penetrated into the cancellous bone, the periosteal graft was encircled by a Nucleopore-filter® (Figure 1). This filter with a pore size of 0.4 micron is impenetrable to cells and vessels (Series 1, comprising 48 joints). In Series 2 (comprising 48 joints) the defects consisted of a superficial part, and a deep part penetrating into the cancellous bone (Figure 2). The animals were allowed normal function immediately after the operation. In 8 rabbits, used as a control group, the operation was the same except that the defect was left without a graft.

Equal numbers of animals were killed 1, 2, 4 and 8 weeks postoperatively. The histological specimens were

0001-6470/82/020181-06 $02.50/0

Figure 1. Schematic presentation of the method. The Nucleopore filter® is placed between the periosteal graft and the subchondral bone.

Figure 2. Schematic presentation of the method showing the superficial part of the defect and the deep part penetrating into the subchondral bone.

prepared using the same method as in a previous study (Rubak 1982).

RESULTS

Series 1

1 week. A grey soft tissue partially filled the defect. Microscopically, there was vigorous proliferation of fibroblast cells in the graft but not on the subchondral side. The fibrous layer of periosteum was seen on the surface (Figure 3).

2 weeks. There was a smooth and somewhat bulging surface of grey glistening tissue. Microscopically a continuous proliferation of fibroblasts and chondroblast-like cells was found on the graft side of the filter. There was no sign of vascularization or bone formation in the area (Figure 4).

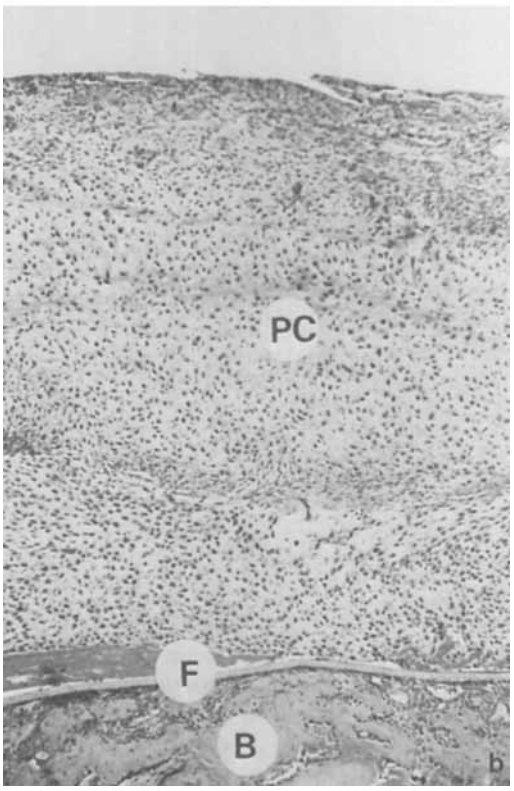

Figure 3. a) Histological picture of the periosteal graft after 1 week. The graft is surrounded by the Nucleopore filter® (F). In this section a connective tissue-like pannus is covering the graft. (H–E, × 25)
b) Detail from a transplantation area after 1 week. Vigorous proliferation of periosteal cells in the graft (PC). Filter (F). Subchondral bone (B). (H–E stain, × 125)

4–8 weeks. A white glistening tissue filled the defect. The tissue comprised young cartilage with a high cell density. The filter was broken, probably because of the vigorous proliferation of the

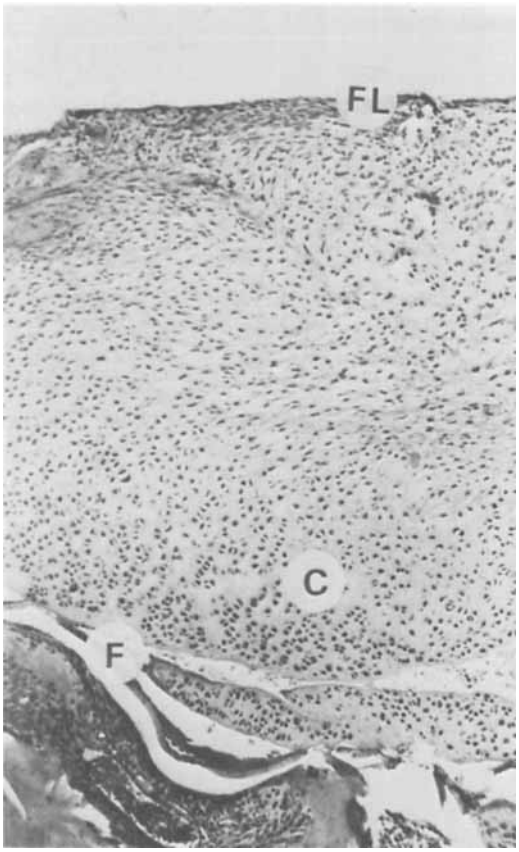

Figure 4. Histological picture from the transplantation area after 2 weeks. The fibrous layer is seen on the surface (FL). In the deeper part of the graft towards the filter (F) cartilage cells are observed (C). (H–E stain, × 125)

underlying bone tissue. The base of the defect was filled with this bone tissue, but the upper part consisted of cartilage-like tissue at the same level as the normal articular cartilage (Figure 5).

Series 2

1–2 weeks. A grey soft tissue partially filled the superficial and the deep part of the defect. Microscopically, proliferation of fibroblasts and chondroblast-like cells was observed (Figure 6).

4–8 weeks. A white glistening tissue filled the defect. Microscopically the tissue was young cartilage. In the superficial part of the defect, however, there was less regenerated cartilage tissue

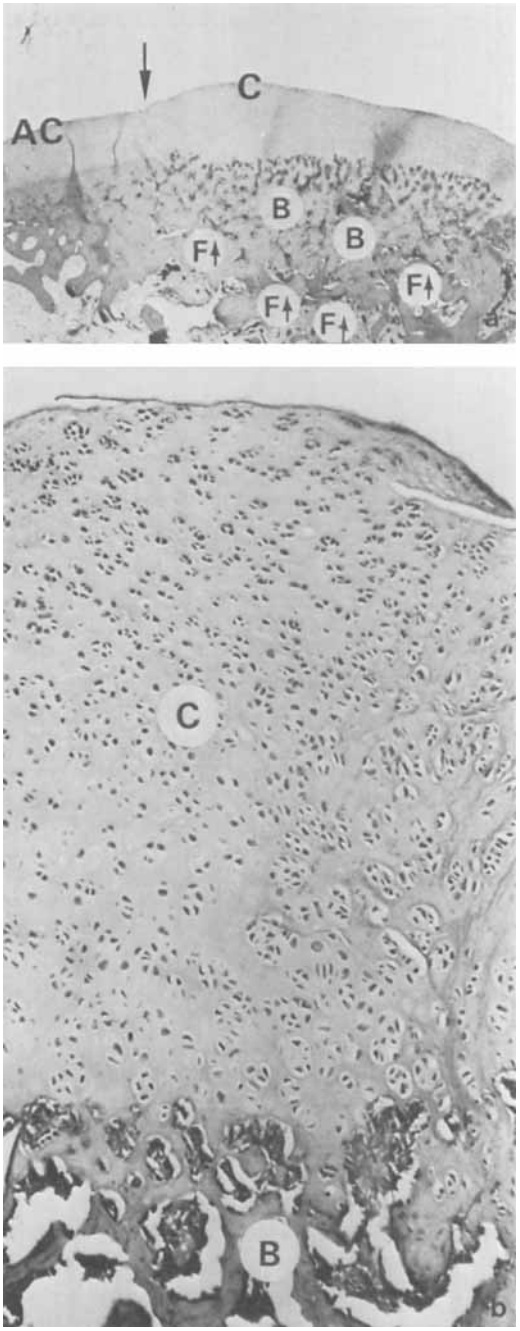

Figure 5. a) Histological picture of the transplantation area after 8 weeks. Normal articular cartilage (AC) and borderline (arrow) to the new generated cartilage (C). The broken filter (F) is observed in the new bone tissue (B). (H–E stain, × 25)
b) Detail of the generated cartilage (C) and bone tissue (B). (H–E stain, × 125)

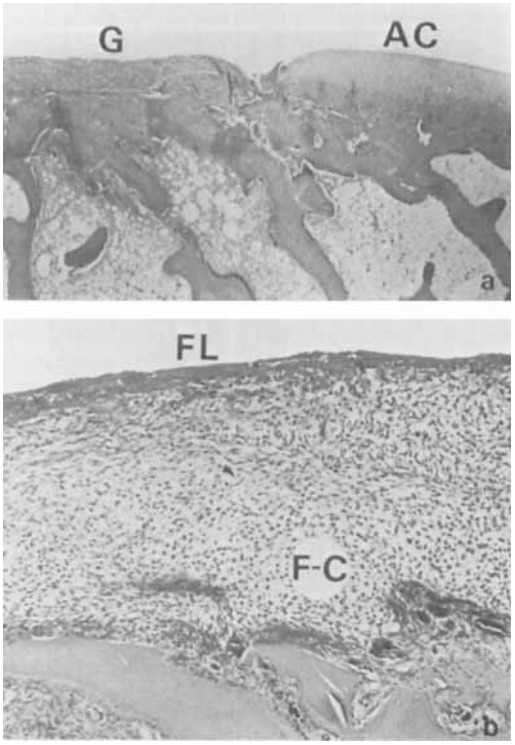

Figure 6. a) Histological picture of the superficial part of the defect after 2 weeks. Normal articular cartilage (AC) on the right and generated tissue (G) on the left. (H–E stain, × 25)
b) Detail of the generated tissue. The fibrous layer is seen on the surface (FL). At a deeper level, proliferation of fibroblasts and chondroblast-like cells is observed. (F–C). (H–E stain, × 125)

and it was of an inferior quality compared with that in the deeper part of the defect. In some experiments the graft had been torn away from the superficial part (Figure 7).

In the control group without grafting there was only a slight tendency towards cartilage regeneration in the deep part of the defect (Rubak 1981). Usually the defect was covered by fibrocartilaginous or fibrous tissue. In the superficial part, the denuded bone surface was found to be without repair tissue or only partially covered with granulation tissue.

DISCUSSION

A previous study has demonstrated the chondrogenic capacity of free periosteal grafts used in the reconstruction of articular cartilage defects (Rubak 1981). However, the question about the origin of the cells which proliferate and repair the defect could not be answered with certainty in that study.

Many investigators have studied the process of repair of articular cartilage defects and the source of the repairing tissue. Redfern (1851) concluded that defects in articular cartilage healed by ingrowth of fibrous tissue which, he believed, arose from the chondrocytes of the articular cartilage.

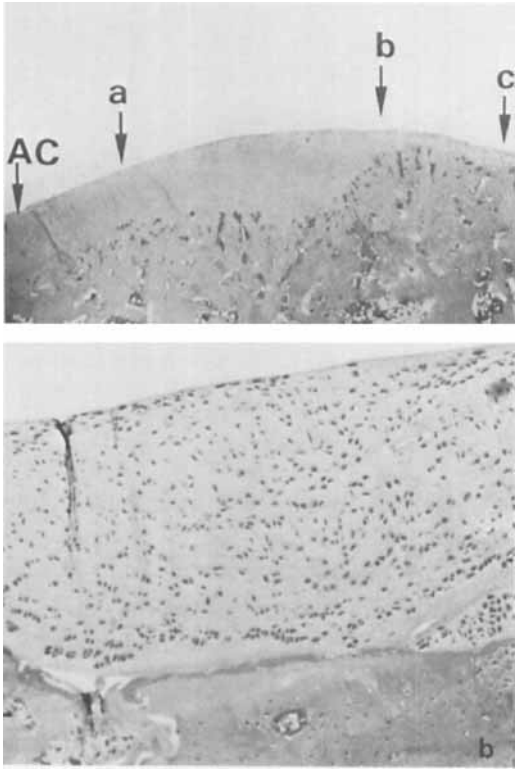

Figure 7. a) Histological picture of the grafted defect after 8 weeks. Normal articular cartilage (AC), the deep part of the defect between the arrows a–b, and the superficial part of the defect between the arrows b–c. (H–E stain, × 25)
b) Detail of the generated tissue, in the superficial part of the defect, comprising a mixture of hyaline and fibrous cartilage. (H–E stain, × 150)

Later on different opinions were put forward as to the origin of the proliferating cells: subchondral bone marrow, synovial membrane, articular cartilage (Haebler 1925, Shands 1931, Calandruccio & Gilmer 1962). The depth of the defect was found to be of great importance, the most active repair was found in defects penetrating to the subchondral bone. DePalma et al. (1966), Campbell (1969) and Mitchell & Shephard (1976) observed filling of deep defects with immature cartilage derived from the subchondral granulation tissue, with the following sequence of events in the development: fibrin, granulation tissue, connective tissue, fibrocartilage and hyaline cartilage (Shands 1931).

In the present study a cell-impenetrable filter was placed in the defect between the cancellous bone and the graft. The defects became filled with cartilage developed directly, without intermediate stages, from mesenchymal cells of the periosteal graft and the tissue maintained cartilaginous morphology. The base of the deep defect was filled with bone tissue supporting the articular layer, but the regenerated cartilage was of the same thickness as the surrounding articular cartilage. The reason that the articular layer did not undergo bony replacement could have been the functional demand for a cartilage covered surface. The phenomenon was also observed by Meachim & Roberts (1971) who studied the healing of drill-holes through the subchondral plate. They found that the repair tissue in the base of the defect was progressively replaced by bone, but not in the surface region. The tissue here comprised fibrous tissue, chondroid tissue and a focal plaque of cartilage. In the present study, however, a continuous layer of cartilage tissue covered the upper part of the defect. Deeper down the filter had disintegrated after 4–8 weeks probably by the force of the proliferating bone tissue. It was not possible to determine whether this ossifying tissue originated from the graft or the cancellous bone.

Cheung et al. (1978) studied the repair tissue in three types of defects: 1) superficial, 2) deep, but no penetration of the subchondral plate, 3) drill-holes deep into the subchondral bone. They found that only in group 3 were the defects filled with hyaline cartilaginous material. The same observation was made by DePalma et al. (1966). In the present study concerning free periosteal grafts, proliferation of cartilage tissue was found in both the deeper part of the defect, and in the part not penetrating to the subchondral bone, although in some cases the proliferation occurred to a lesser extent in the superficial part of the defect. This may be due to mechanical wear, which is greater for the superficial part of the graft. Better proliferation of the graft might be achieved if the joint was immobilized for a few days to ensure a firmer attachment of the graft in the recipient area. Furthermore it is possible that the graft in the deep part of the defect may receive some nutrition from the subchondral bone area, in addition to that from the synovial fluid, and this may result in better proliferation.

In the control group only the deep part of the defect was partially filled with connective tissue, fibrocartilage or occasionally bone. The fact that these large defects were not filled with cartilage, as in studies of DePalma et al. (1966), Campbell (1969), Salter et al. (1975), Mitchell & Shephard (1976), could be explained by the different size of the defects. The findings of Convery et al. (1972) are in accordance with the present study. They studied healing of defects of different sizes (3–21 mm) penetrating the subchondral plate in the femoral condyle of ponies. None of the large defects were completely filled with repair tissue, which comprised a mixture of fibrous tissue, fibrocartilage, hypercellular cartilaginous tissue and occasionally bone. Campbell (1969) also found healing to be most complete in small defects.

According to the present investigation the cartilage formed in the defects originates from the cells of the periosteal grafts and not from the subchondral bone cells. How chondrogenesis is regulated is not clear. Mechanical factors such as compression and motion are probably of great importance. Other factors influencing the process could be the synovial fluid or diffusion of chemical substances from subchondral bone. Factors promoting the chondrogenesis in free periosteal grafts will be studied in further investigations to clarify the clinical applications of the method.

REFERENCES

Bennett, G. A. & Bauer, W. (1932) A study of the repair of articular cartilage and the reaction of normal joints of adult dogs to surgically created defects of articular cartilage, "joint mice" and patellar cartilage. *Am. J. Pathol.* **8**, 499–523.

Calandruccio, R. & Gilmer, W. (1962) Proliferation, regeneration and repair of articular cartilage of immature animals. *J. Bone Joint Surg.* **44**-A, 431–455.

Campbell, C. (1969) The healing of cartilage defects. *Clin. Orthop.* **64**, 45–63.

Carlson, H. (1957) Reactions of patellary cartilage following operative defects. *Acta Orthop. Scand.*, Suppl. 28.

Cheung, H., Cottrell, W. H., Stephenson, K. & Nimni, M. E. (1978) In vitro collagen biosynthesis in healing and normal rabbit articular cartilage. *J. Bone Joint Surg.* **60**-A, 1076–1081.

Convery, R., Akeson, W. & Keown, G. H. (1972) The repair of large osteochondral defects. *Clin. Orthop.* **82**, 253–262.

DePalma, A., McKeever, D. & Subin, D. K. (1966) Process of repair of articular cartilage demonstrated by histology and autoradiography with tritiated thymidine. *Clin. Orthop.* **48**, 229–242.

Haebler, C. (1925) Experimentelle Untersuchungen über die regeneration des Gelenkknorpels. *Beitr. Klin. Chir.* **134**, 602–640.

Mankin, H. J. (1962) Localization of tritiated thymidine in articular cartilage of rabbits II. Repair in immature cartilage. *J. Bone Joint Surg.* **44**-A, 688–698.

Meachim, G. & Roberts, C. (1971) Repair of the joint surface from subarticular tissue in rabbit knee. *J. Anat.* **109**, 2, 317–327.

Mitchell, N. & Shephard, N. (1976) The resurfacing of adult rabbit cartilage by multiple perforations through the subchondral bone. *J. Bone Joint Surg.* **58**-A, 230–233.

Redfern, P. (1851) On the healing of wounds in articular cartilage. *Month. J. Med. Sci.* **13**, 201–215.

Rubak, J. M. (1982) Reconstruction of articular cartilage defects with free periosteal grafts. *Acta Orthop. Scand.* **53**, 175–180.

Sakakida, K., Inoul, S. & Maki, Y. (1978) An experimental study of the repair of injured articular cartilage using ^{3}H Thymidine autoradiography. *Int. Orthop.* **2**, 3–7.

Salter, R. B., Simmons, D. F., Malcolm, B. W., Rumble, E. J. & MacMichael, D. (1975) The effects of continuous passive motion on the healing of articular cartilage defects. *J. Bone Joint Surg.* **57**-A, 570–571.

Shands, A. R. (1931) The regeneration of hyaline cartilage in joints. *Arch. Surg.* **22**, 137–178.

Correspondence to: Jens M. Rubak, M.D., Engdalgårdsvej 6, 8330 Beder, Denmark.