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RECONSTRUCTION OF ARTICULAR CARTILAGE DEFECTS WITH FREE PERIOSTEAL GRAFTS

An Experimental Study

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The chondrogenic potential of free autogenous periosteal grafts was studied histologically in 6-month-old rabbits. The grafts were taken from the tibia and transplanted to 7 × 14 mm large artificial defects of the femoral articular cartilage. The results revealed that the defects were repaired and filled after 4 weeks with a hyaline-like cartilage which was histologically similar to the cartilage adjacent to the transplant. The tissue maintained this morphology after 1 year of observation. In control animals where no periosteum was transplanted to the defect, no real cartilage was found. The tissue which partially filled the defect was a variable mixture of fibrous tissue and fibrocartilage.

Key words: articular cartilage repair; chondrogenesis; periosteal graft

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The osteogenic capacity of periosteum has been known since the classical studies of Duhamel (1739) and Ollier (1867). In more recent studies by Ritsilä et al. (1972) cartilage formation was often found to precede the bone formation from free periosteal grafts. It is possible that the cambium layer cells of periosteum possess a dual potentiality of differentiation, depending on the environmental conditions (Ham 1930).

The purpose of this study, with special reference to clinical design, is to examine whether the cartilaginous environment of joints is able to direct the line of differentiation in the periosteal cells into chondroblasts and chondrous tissue and in this way restore the cartilage in articular cartilage defects.

MATERIAL AND METHODS

The experimental material comprised 132 knee joints in 66 rabbits weighing between 2500 and 3300 g. The animals were housed in wire mesh cages and received food pellets and water *ad libitum*. The operations were performed under anaesthesia with Nembutal i.v. as induction and continued with Halothane, N_2O/O_2. On the proximal part of the medial facet of the tibia the periosteum was exposed and removed by stripping. The knee joint was opened by a medial parapatellar incision and the patella was dislocated laterally. Articular cartilage was resected with a chisel down into bleeding subchondral bone as shown in Figure 1. The size of the defect was 7 × 14 mm. The periosteal graft was trans-

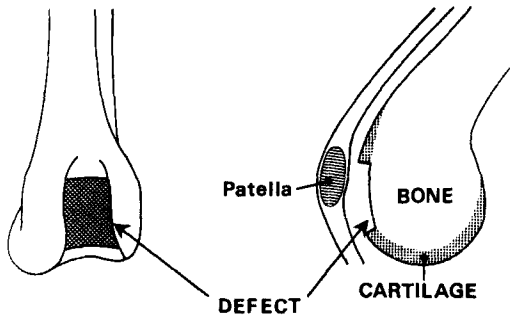

Figure 1. Schematic presentation of the method. The periosteal graft is transplanted to the defect.

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planted to the defect with the cambium layer facing the spongiosa and sutured with 6–0 Vicryl® to the synovial membrane. The joint was closed in two layers and the rabbit was allowed normal mobility immediately after the operation.

The animals were killed 4, 7, 14, 21, 28, 84, 180 and 360 days postoperatively, and there were respectively, 16, 16, 16, 12, 16, 12, 12, and 8 joints in the various groups. In 12 animals used as a control group the operative procedure was the same except that the denuded subchondral bony surface was left without a graft. These animals were killed after 4, 7, 14, 21, 28 and 42 days and there were 4 joints in each group. The specimens were fixated in formaldehyde, decalcified in formic acid, dehydrated in serial alcohol concentrations, cleared in xylene and embedded in paraffin. Sections were cut to 2–5 micron thickness and stained with haematoxylin eosin and van Gieson.

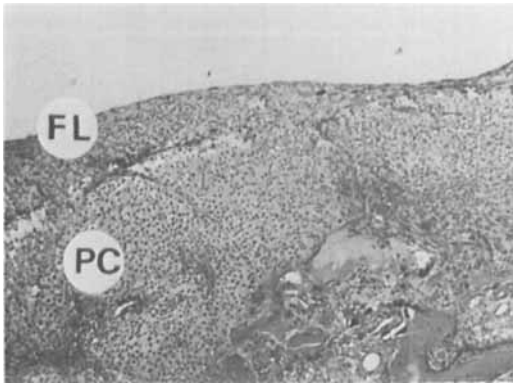

Figure 2. Histological picture of the transplantation area after 7 days. The fibrous layer (FL) of periosteum is seen on the surface. Vigorous proliferation of periosteal cells (PC). (H–E stain, × 125).

RESULTS

Macroscopic findings

Two of the rabbits in the grafted group died 6 days after the operation. No sign of infection was detected but the animals were excluded from the series. The rest of the animals showed no sign of discomfort and reached a weight of 4000–5600 g after 3 to 12 months. There was no difference in mobility in the grafted and the control group. The knee joint was often found to be thickened. In the grafted group, the defect was covered, during the first 2 weeks, by a grey fibrous-like tissue. Later it was covered by a white glistening cartilage-like tissue resembling the normal surrounding articular cartilage. There seemed to be a well-healed borderline between the original cartilage and the new cartilage-like tissue.

In the control group, the defect was partially filled with a grey soft tissue, which did not reach the level of the articular surface. No true repair of cartilage was found.

Microscopic findings

4–7 days. A marked proliferation of mesenchymal cells in the transplantation area could be observed. The fibrous layer of the periosteum could be seen on the surface (Figure 2).

14 days. Continuous and more vigorous proliferation of fibroblast cells and chondroblast-like cells was seen. The defect was filled with the proliferating tissue, and a smooth bulging of the surface in the transplantation area was seen (Figure 3).

21–28 days. The tissue formed in the defect had an appearance similar to young cartilage. An increased amount of matrix separated the chondrocyte-like cells. Some cells were situated in typical cartilage lacunae (Figure 4).

42 days. No enchondral ossification had occurred. The cell density was higher in the transplantation area than in the surrounding

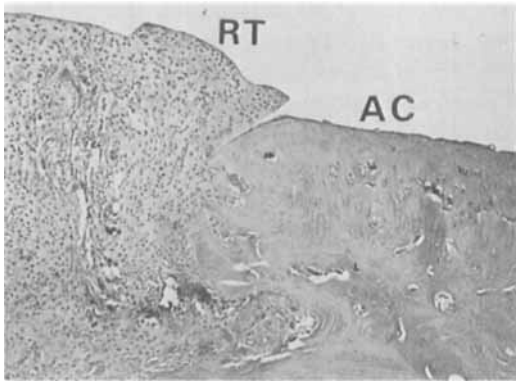

Figure 3. Histological picture of the transplantation area after 14 days. The regenerated tissue to the left side (RT) has proliferated above the original articular surface (AC) to the right side. Chondroblast-like cells can be observed. (H–E stain, × 125).

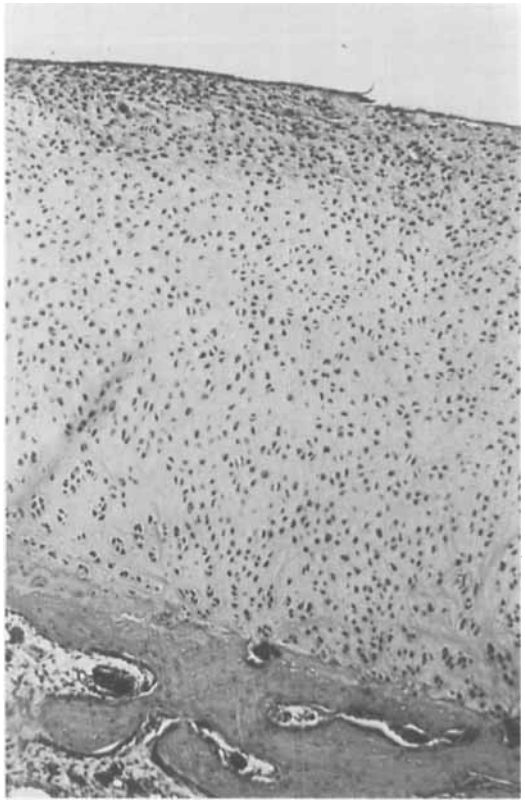

Figure 4. Histological picture of the transplantation area after 28 days. The tissue has the appearance of immature cartilage. Some chondrocytes are seen in lacunae. The chondrocytes are separated by an increased amount of matrix. The surface is even and there is a distinct borderline to the subchondral bone. No bone formation in the transplantation area. (H–E stain, × 125).

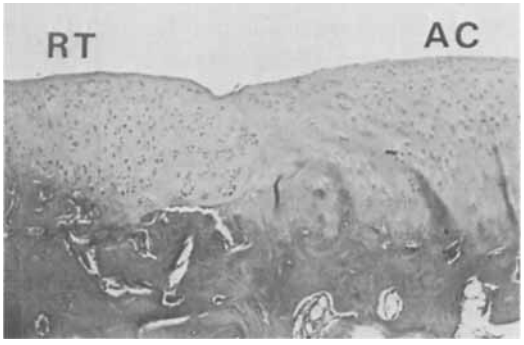

Figure 5. Histological picture of the transplantation area after 180 days. The cell density has decreased and the generated tissue (RT) to the left more closely resembles normal articular cartilage. Some formation of palisades and lacunae can be observed. Normal articular cartilage (AC) to the right. (H–E stain, × 125).

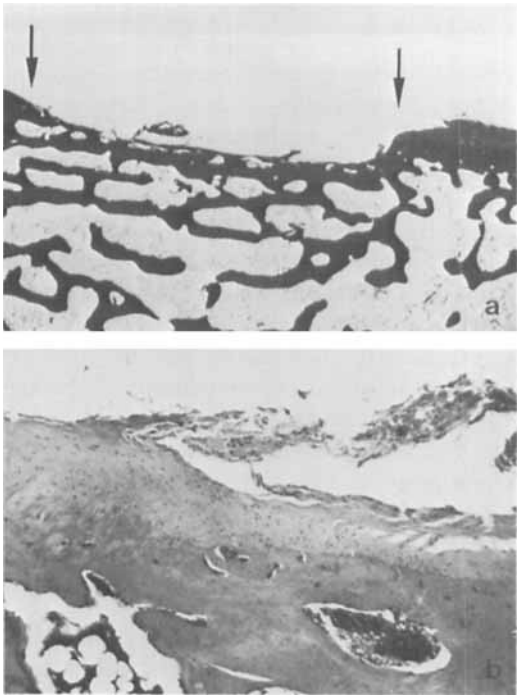

Figure 6. a) Histological picture of the defect in the control group after 42 days. The defect between the arrows is clearly visible. The edges are somewhat smoothened and rounded. (v–G stain, × 25). b) In higher magnification a thin fibrous or fibrocartilaginous tissue layer is seen partially filling the defect. (H–E stain, × 125).

original cartilage, and the pattern was more irregular. The borderline between the transplantation area and the articular cartilage was in some instances filled with connective tissue, but for the most part it was disappearing at this stage.

84–360 days. The tissue formed in the transplantation area resembled increasingly the adjacent hyaline cartilage. The cell density had decreased. On the surface there were flattened elongated cells; under these the cells were larger and a tendency to vertical palisade formation was observed. In most experiments the surface was smooth and did not show degenerative alterations. There was a well-defined borderline-zone to the subchondral bone (Figure 5).

In the control group there was only a slight tendency towards cartilaginous regeneration. Mostly the defect was covered by fibrous tissue without cartilage or bone formation, and was seldom filled completely (Figure 6).

DISCUSSION

Previous studies have demonstrated the capacity of free periosteal grafts from long bones to form bone either by enchondral ossification, i.e. through the cartilage stage, or directly by intramembranous ossification (Ritsilä et al. 1972, Alhopuro 1978). Ham (1930) mentioned the possibility of the cells in the cambium layer of the periosteum differentiating into chondroblasts depending on environmental conditions during the embryonic stage, and that certain cells persist in the adult organism with the potency of these undifferentiated mesenchymal cells (Ham 1953). The effect of the environment on the line of differentiation of the periosteal cells has been further observed in studies of Haas (1914), Berg & Thalheimer (1918), Klinkerfuss (1924), Cohen & Lacroix (1955), Ritsilä & Alhopuro (1973), Poussa et al. (1981) and Rubak (1980).

In the present study the articular cartilage defects became filled with a hyaline-like cartilage, with matrix separating the cells, obviously generated from the cambium layer of the periosteal graft. There was no vascularization in the area and no total enchondral ossification occurred as in studies where periosteum was transplanted to muscle tissue (Ritsilä et al. 1972, Poussa et al. 1980). However, in nine experiments ossifying processes occurred in the transplantation area, but did not proceed to the surface. Various factors can be responsible for this. Basset (1962) demonstrated that high oxygen pressure in culture media resulted in bone formation, and that cartilage formation occurred in media with low oxygen pressure. In the present study the removal of too much cancellous bone in the recipient area might have promoted better vascularization giving a higher oxygen pressure with enchondral ossification as a result. Also a strong osteogenetic stimulus from the cancellous bone might favour enchondral ossification. In control animals there was no real cartilage regeneration.

Various attempts have been made to restore the articular surface with non-cartilaginous tissue. For example, Murphy (1905) used fat and fascia, Sumita (1912) muscle, fat and fascia, Loewe (1929) dermal grafts, Perigalli (1951) skin grafts and De Marchi & Cambier (1953) skin grafts as interposition material. These studies revealed a fibrous transformation of the interposition material, but not to hyaline-like cartilage. In arthroplasties on cats with skin grafts Kettunen (1958) states that he demonstrated functional metaplasia into cartilage in the interposition material; he also observed the occurrence of ossifying processes. Hofmann (1908) used periosteum and observed formation of bone on the subchondral surface. The graft he used might have been osteo-periosteal, which is different from the grafts used in the present study. In 1972 Skoog et al. showed the chondrogenic capacity of free perichondrial grafts. Free perichondrial grafts were used in experimental reconstruction of articular cartilage defects by Ritsilä & Alhopuro (1975) and Engkvist (1979).

Some writers believed the various interposition materials to be of secondary importance, only acting as a culture medium within which chondrogenesis may occur (Hoover & Coventry 1961). Salter et al. (1975) reported regeneration of cartilage from the subchondral bone in small drill holes in the femoral head of rabbits. Meachim & Roberts (1971), after removal of the articular cartilage, drilled holes in subchondral bone and observed regeneration of fibrocartilaginous tissue. In 1972, Convery et al. found the repair tissue in large defects, like those in the control animals in the present study, to comprise fibrous tissue, fibrocartilage, hypercellular cartilage and occasionally bone.

The appearance of hyaline-like cartilage tissue after periosteal grafting in the present study can be due to various other factors, in addition to the chondrogenic differentiation potential of the periosteal cells. The synovial fluid is an adequate source of nutrition for cartilage proliferation as shown by Strangeways (1920), Mankin (1963), Maroudas et al. (1968), Zahir & Freeman (1972), Ogata & Whiteside (1979), and it may contain chondrotrophic properties. The theory of a chemical stimulus for differentiation of cells is supported by the studies of Urist et al. (1967). The importance of articular loading and function as factors contributing to the formation of cartilage has been demonstrated in studies of Mooney & Ferguson (1966), Salter et al. (1975), Mitchell & Shepard (1976). This was likewise confirmed

in the present study, as it seemed that the most advanced stage of hyaline-like cartilage tissue was found in the areas bearing the most weight in the centre of the defect.

After comparing the results of earlier free periosteal grafting in different environments with the results of this study, it is obvious that the environment influences the differentiation of the periosteal cells. Further investigations are in progress to elucidate these environmental factors and the exact origin of the hyaline-like cartilage tissue. The findings suggest the possibility of clinical applications of the method.

REFERENCES

Alhopuro, S. (1978) Premature fusion of facial sutures with free periosteal grafts. *Scand. J. Plast Reconstr. Surg.,* Suppl. 17.

Basset, C. A. L. (1962) Current concepts of bone formation. *J. Bone Joint Surg.* **44–A**, 1217–1244.

Berg, A. & Thalheimer, W. (1918) Regeneration of bone. *Ann. Surg.* **67**, 331–347.

Cohen, J. & Lacroix, P. (1955) Bone and cartilage formation by periosteum. *J. Bone Joint Surg.* **37**-A, 717–730.

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

De Marchi, R. & Cambier, R. (1953) Ricerche istologiche sperimentali e cliniche sull arthroplastica con cute. *Minerva Ortop.* **4**, 29–35.

Duhamel, H. L. (1739) cited by Basset, C. A. L. in Current concepts of bone formation. *J. Bone Joint Surg.* **44**-A, 1217–1244.

Engkvist, O. (1979) Formation of articular cartilage from free perichondrial grafts. *Acta Univ. Uppsala* 322.

Ham, A. W. (1930) A histological study of the early phases of bone repair. *J. Bone Joint Surg.* **12**, 827–844.

Ham, A. W. (1953) *Histology.* 2nd Ed. Lippincott, London.

Haas, S. L. (1914) Regeneration of cartilage and bone with a special study of these processes as they occur at the chondrocostal junction. *Surg. Gynecol. Obstet.* **19**, 604–617.

Hofmann, M. (1908) Weitere Unterzuchungen und Erfahrungen über Periosttransplantation bei Behandlung knöcherner Gelenkankylosen. *Bruns' Beitr. Klin. Chir.* **59**, 717–732.

Hoover, N. W. & Coventry, M. B. (1961) Skin arthroplasty of the hip. *J. Bone Joint Surg.* **43**-A, 1155–1166.

Kettunen, K. O. (1958) Skin arthroplasty in the light of animal experiments with special reference to functional metaplasia of connective tissue. *Acta Orthop. Scand.,* Suppl. 29.

Klinkerfuss, G. (1924) A study of the growing power of periosteal callus when transplanted to costal cartilage. *Surg. Gynecol. Obstet.* **38**, 625–631.

Loewe, O. (1929) Ueber Haut-Tiefenplastik. *Münch. Med. Wochenschr.* **76**, 2125–2128.

Mankin, H. J. (1963) Localization of tritiated cytidine in articular cartilage of immature and adult rabbits after intra-articular injection. *J. Lab. Invest.* **12**, 543–548.

Maroudas, A., Bullough, P. Swanson, S. A. V. & Freeman, M. A. R. (1968) The permeability of articular cartilage. *J. Bone Joint Surg.* **50**-B, 166–177.

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

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

Mooney, V. & Ferguson, A. B. (1966) The influence of immobilization and motion on the formation of fibrocartilage in the repair granuloma after joint resection in the rabbit. *J. Bone Joint Surg.* **48**-A, 1145–1155.

Murphy, J. B. (1905) Anchylosis, arthroplasty, clinical and experimental. *JAMA* **44**, 1573–82, 1671–78, 1749–56.

Ogata, K. & Whiteside, L. (1979) Barrier to material transfer of the bone – cartilage interface. *Clin. Orthop.* **145**, 273–276.

Ollier, L. (1867) *Traité experimental et clinique de la regeneration des os et de la production artificielle du tissu osseaux.* Vol. I, Masson & Fils Paris.

Peregalli, P. (1951) Richerche sperimentali sull' arhtroplastica con interposizione di lembi cutanei. *Arch. Ortop. (Milano)* **64**, 16–33.

Poussa, M., Rubak, J. M. & Ritsilä, V. (1980) The effect of the thickness of the cortical bone on bone formation by osteoperiosteal grafts. *Acta Orthop. Scand.* **51**, 29–35.

Poussa, M., Rubak, J. M. & Ritsilä, V. (1981) Differentiation of the osteochondrogenic cells of the periosteum in chondrotrophic environment. *Acta Orthop. Scand.* **52**, 235–239.

Ritsilä, V., Alhopuro, S. & Rintala, A. (1972) Bone formation with free periosteum. *Scand. J. Plast. Reconstr. Surg.* **6**, 51–56.

Ritsilä, V. & Alhopuro, S. (1973) Reconstruction of experimental tracheal cartilage defects with free periosteum. *Scand. J. Plast. Reconstr. Surg.* **7**, 116–119.

Ritsilä, V. & Alhopuro, S. (1975) Regeneration of articular cartilage defects with free perichondrial grafts. *JRCS Med. Sci. Connective Tissue, Skin and Bone; Surgery and Transplantation* **3**, 49–51.

Rubak, J. M. (1980) Reconstruction of the experimental condylar cartilage defect of the knee joint with free periosteal grafts, Paper read at the XL th Congress of the Scand. Orthop. Assoc. June, 1980, Helsinki.

Salter, R. B., Simmonds, 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.

Skoog, T., Ohlsen, L. & Sohn, S. A. (1972) Perichondrial potential for cartilaginous regeneration. *Scand. J. Plast. Reconstr. Surg.* **6**, 123–125.

Strangeways, T. S. P. (1920) The nutrition of articular cartilage. *Br. Med. J.* **15**, 661–665.

Sumita, M. (1912) Experimentelle Beiträge zur operativen mobilisierung ankylosierter Gelenke. *Arch. Klin. Chir.* **99**, 756–815.

Urist, M. R., Silvermann, B. G. Buring, K. Dubuc, F. L. & Rosenberg, J. M. (1967) The bone induction principle. *Clin. Orthop.* **53**, 243–283.

Zahir, A. & Freeman, M. A. R. (1972) Cartilage changes following a single episode of infarction of the capital femoral epiphysis in the dog. *J. Bone Joint Surg.* **54**-A, 125–136.

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