Acta orthop. scand. 52, 235–239, 1981

DIFFERENTIATION OF THE OSTEOCHONDROGENIC CELLS OF THE PERIOSTEUM IN CHONDROTROPHIC ENVIRONMENT

MIKKO POUSSA, JENS RUBAK & VEIJO RITSILÄ

The Orthopaedic Hospital of the Invalid Foundation, Helsinki, Finland, and Department of Orthopaedic Surgery, Århus County Hospital, Århus, Denmark

The behaviour of the cells in free periosteal grafts was studied in growing rabbits in three chondrotrophic recipient milieus: costal cartilage, ear cartilage and synovial fluid of the knee joint. The periosteal grafts first formed cartilage, which was then quite rapidly transformed into bone on the costal cartilage, quite slowly and in smaller amounts in the ear cartilage, whereas no bone was found in the cartilaginous loose bodies formed in the knee joint. In bone formation vascularization plays a major role, but other factors are also involved.

Key words: chondrotrophic environment; differentiation; osteochondrogenic cell; periosteal graft; proliferation

Accepted 2.x.80

The development of vascularization is considered mandatory for osteogenesis (Trueta 1963, Albrektsson 1980). This conception was confirmed in studies with periosteal grafts in muscle tissue (Poussa 1980). However, other factors are also essential for formation of new bone, but these are less known and thought to be humorally mediated or based on close interactions between the graft and the recipient bed cells (Urist et al. 1967, Saxen 1972). On the other hand some experimental and clinical findings show that periosteal grafts on a joint surface can form cartilage-like tissue (Ritsilä et al. 1979). The purpose of the present study was to examine in detail the effect of the various chondrotrophic environments on the proliferation of periosteal cells.

MATERIAL AND METHODS

Twenty-five rabbits aged 6 to 8 weeks were operated on. Periosteum was taken from the medial side of both tibiae by stripping. These periosteal grafts were then transplanted (Figure 1) onto costal cartilage, where the perichondrium had been scraped off (Series I); onto the ear cartilage beneath the perichondrium (Series IIA) as well as into the ear between the two layers of skin, where the cartilage with both layers of perichondrium had been removed (Series IIB), and into synovial fluid of the knee joint, where the periosteal grafts were implanted as loose bodies (Series III). In some experiments of Series I, a Nucleopore® filter (pore diameter

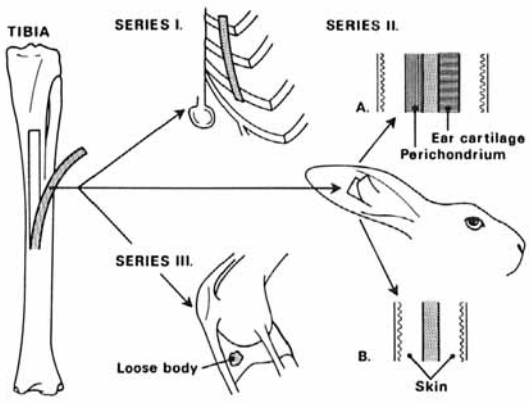

Figure 1. Schematic presentation of the material. Tibial periosteum was transplanted to three beds: the costal cartilage (Series I), the ear between the perichondrium and cartilage (Series IIA) or between the two skin layers (Series IIB), the synovial fluid of the knee joint (Series III).

0001-6470/81/030235-05 $02.50/0

0.4 μ) was fixed between the costal cartilage and the periosteal graft. On the costal and ear cartilages, the periosteum was grafted so that the cambium layer faced the cartilage of the recipient bed.

The rabbits were killed 1, 2, 3, 6 and 8 weeks after the transplantation. The samples were fixed in 10 per cent neutral buffered formalin, decalcified in formic acid, cleared in xylene and toluene, embedded in paraffin and stained with haematoxylin eosin, van Gieson and alcianblue. With alcianblue the acidic glycosaminoglycans give a blue colour which histologically indicates newly formed cartilaginous tissue.

RESULTS

Series I

At 1 week, proliferation of the cambium layer cells and the presence of osteoid-like tissue between the cells. No formation of cartilage or bone.

At 2 weeks, cancellous bone formation by the graft, especially in the muscle bed between the costal cartilages. Cartilage discs were also seen on the surfaces of the costal cartilages. In those samples where the Nucleopore® filters were used, cartilage proliferation occurred on both sides (Figure 2).

At 3 to 8 weeks, the bone formed had a haematopoetic marrow, but cartilaginous zones separating the newly formed bone from the costal cartilage were still present (Figure 3).

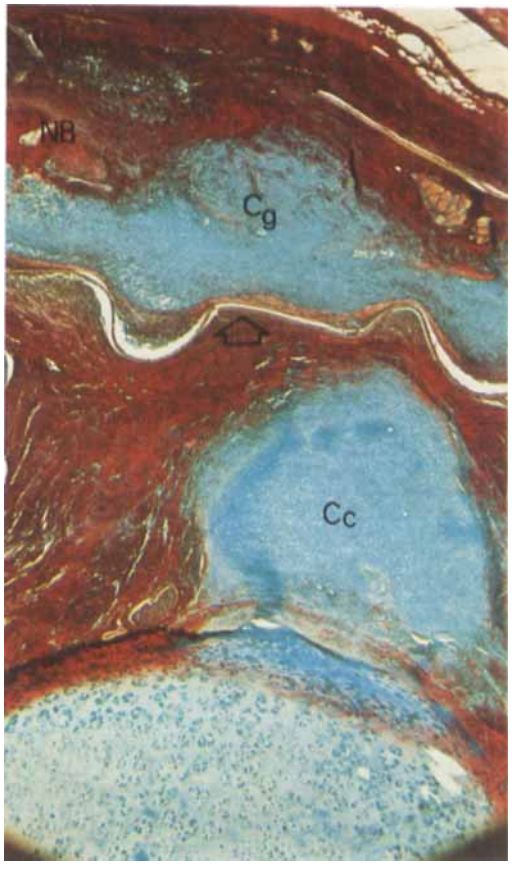

Figure 2. Series I, 14 days after periosteal transplantation onto costal cartilage. Cartilage formation by the graft (Cg) and by the costa (Cc). The Nucleopore® filter is seen between the two cartilage masses (arrow). New bone formation is also present (NB). (Alcian blue, ×50).

Series II

II A

At 1 week, vigorous proliferation of small cartilaginous cells in the transplantation area, no traces of bone formation.

At 2 weeks, vigorous proliferation of small cartilaginous cells, whose origin could not be ascertained.

At 3 weeks, continuing proliferation of cartilaginous cells, no bone formation.

At 6 to 8 weeks, bone formation, but in an insignificant amount, as well as continuing proliferation of cartilaginous cells (Figure 4).

II B

At 1 week, a trabecular bony ossicle was formed between the two skin layers.

At 2 weeks, a bony ossicle almost the same as at 1 week, no traces of cartilage.

At 3 weeks, more mature bone, resembling cortical bone in structure.

At 6 to 8 weeks, fully developed cortical bone.

Series III

At 1 week, proliferation of periosteal cells, the graft still resembling its original irregularly elliptical shape.

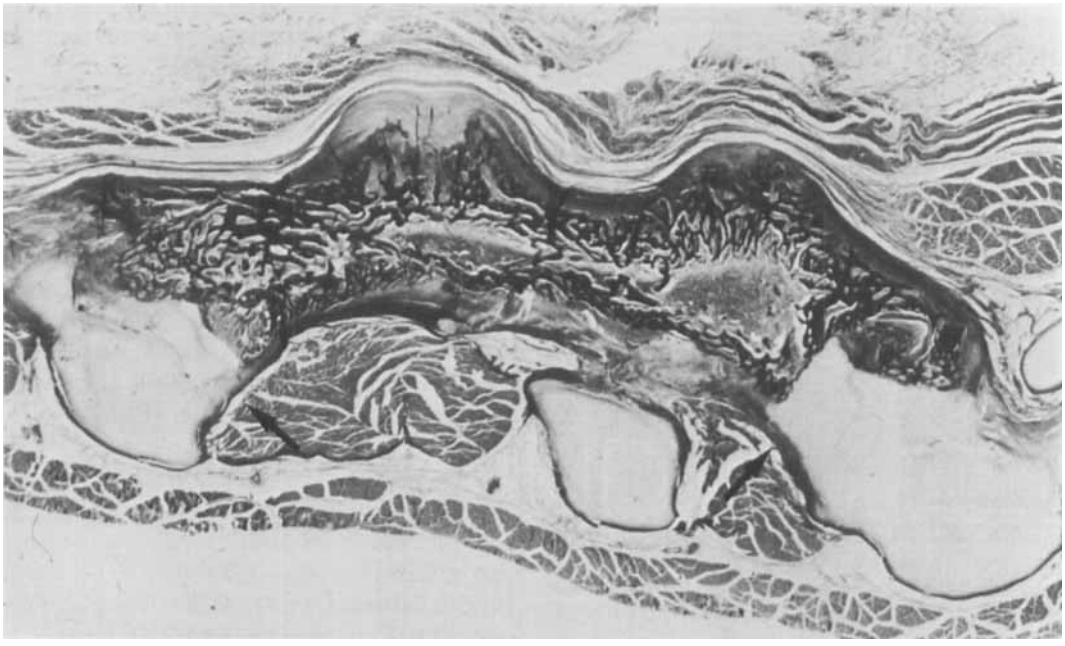

Figure 3. Series I, 42 days after periosteal transplantation on costal cartilage. The bone formed has a haematopoetic marrow, cartilage zones still separating the ossicle from costal cartilage (arrows). (Van Gieson, ×6).

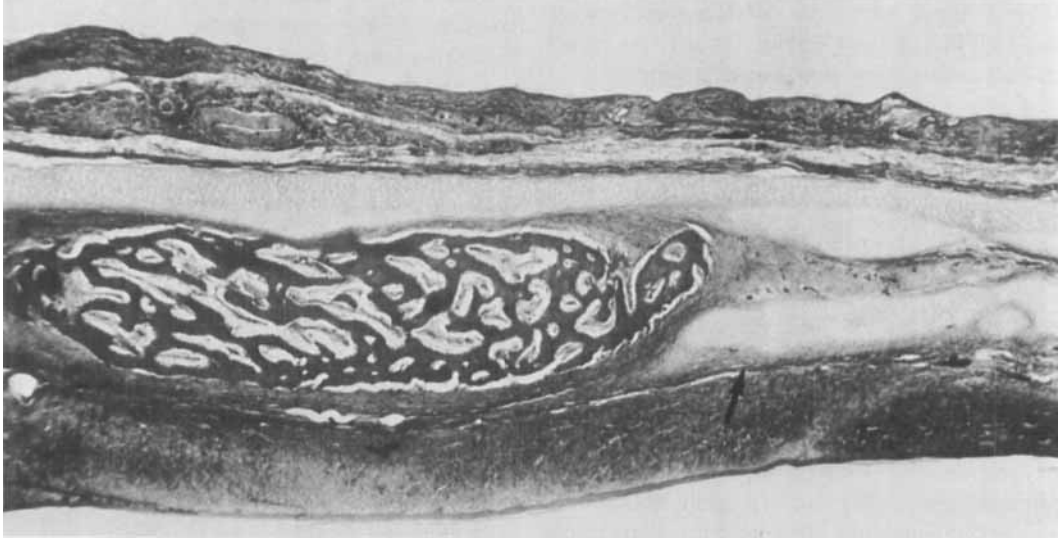

Figure 4. Series IIA, 42 days after transplantation, there is an ossicle between the ear cartilage and the perichondrium. Immature cartilage is still being formed as well (arrow). (Van Gieson, ×40).

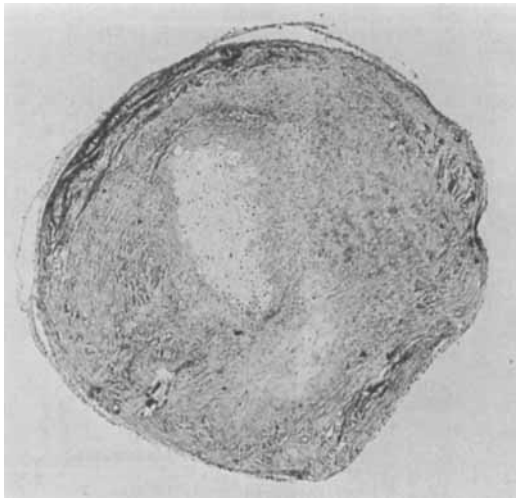

Figure 5. Series III, 14 days after transplantation of free periosteum into knee joint. A spherical loose body has been formed. In its centre hypertrophied cartilage cells are seen surrounded by fibrotic tissue. (Van Gieson, ×40).

At 2 weeks, the transplant had become spherical and large mature chondrocytes resembling secondary cartilage cells were seen in the centre of the graft (Figure 5).

At 3 to 8 weeks, the spherical graft was surrounded by small epithelial cells. In the centre, fibrotic tissue and signs of fatty degeneration were visible. After 4 weeks, these loose bodies tended to disappear from the knee joint.

DISCUSSION

In earlier periosteal transplantation studies (Poussa & Ritsilä 1979, Poussa et al. 1980) bone formation occurred enchondrally, i.e. through a cartilage interphase. This may be due to the rapidity of the proliferation of the primitive osteogenic cells as the vascularization was slower. According to Basset (1962) as well as to Ham & Harris (1971), under the influence of a low oxygen tension there is a tendency for the osteoprogenitor cells to differentiate into fibrotic or cartilaginous cells. Moreover, it is known that during the embryonic period of bone development, the periosteum is derived from perichondrium. During the proliferation of periosteal graft cells in a muscular environment, a repetition of the embryonic differentiation may occur.

Apart from the development of adequate vascularization (Rhinelander 1972), there are other prerequisites for osteogenesis, although these factors are poorly understood. The study of Cohen & Lacroix (1955), in which a tibial periosteal graft formed bone more vigorously on the surface of the tibia than in the kidney parenchyma, demonstrates the importance of these other unknown factors. Also spleen and liver are a poor environment for osteogenesis irrespective of their intensive circulation (Schepelmann 1913).

In the present study, bone formation occurred on the costal cartilage, but it started from the muscular side of the bed. This is in accordance with other studies (Haas 1914, Berg & Thalhimer 1918, Klinkerfuss 1924). Klinkerfuss used fracture callus as graft material for transplantation on costal cartilage. The bone formation was quite rapid, which obviously was due to the good circulation in the muscles and most likely influenced by the osteogenic nature of the costal cartilage. The latter is considered osteogenic as it ossifies with age. Also in our studies the cartilaginous zone developed between the newly formed bone and costal cartilage. It persisted as long as 8 weeks after the transplantation. The zone was produced by the costal cartilage, which was confirmed with the aid of a Nucleopore® filter.

In the ear, a clear difference was observed between the two groups. When periosteum was implanted between the perichondrium and cartilage the differentiation was cartilaginous. Apparently this can be explained by the insufficient circulation in the recipient bed, because its nutrition is obtained by diffusion through the cartilage or perichondrial cells. When periosteum was grafted between the two layers of well vascularized skin a rapid bone formation occurred within 1 week.

The synovial fluid was ideal as an avascular environment. The proliferation of periosteal cells was rapid. Cartilage appeared in loose bodies within 2 weeks and resembled so-called secondary cartilage. Nevertheless, the process stopped here and the hypertrophied cartilage cells rapidly degenerated soon afterwards. The cause of this

cessation of proliferation remained unclear. In tissue culture studies, where the grafted cells are likewise in avascular conditions, the proliferation of undifferentiated mesenchymal cells produced bone (Basset 1962, Ritsilä et al. 1972). Ritsilä et al. (1972) used periosteal grafts in culture media and achieved bone spicules within 4 weeks. It can be assumed that the chondrotrophic environment of the synovial fluid stops the proliferation and differentiation processes of periosteal cells at the chondrous stage so bone was not formed in the joint cavity. This is in accordance with the observations of Hofmann (1908) and Rubak (1980), who used free periosteal grafts to resurface experimentally induced articular cartilage defects in the joints of dogs and rabbits. Hofmann (1908), however did not give an accurate qualitative description. In Rubak's (1980) experiments, hyaline-like cartilage was achieved.

From our results we conclude that free periosteal grafts do not behave in a chondrotrophic milieu in the same way as they do in a muscular bed. In the muscular environment free periosteal grafts consistently formed trabecular bone within 1 week and this rapidly matured into lamellar bone. In the present study the enchondral ossification was very weak and slow and in some series did not occur at all. The poorer vascularity of the bed is one explanation but other local factors also seem to have an effect.

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Correspondance to: Mikko Poussa, The Orthopaedic Hospital of the Invalid Foundation, Tenholantie 10, 00280 Helsinki 28, Finland.