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The development of bone after perichondrial grafting: an experimental study using ear and rib perichondrium in rabbits

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Summary—The development of bone after perichondrial grafting was investigated using rabbit ear and rib perichondrium. Sixty-four white adult female rabbits were used. Both free and vascularised perichondrial grafts were undertaken. In each case the chondrogenic potential of perichondrium was proved. Furthermore, when the perichondrium was vascularised or grafted in recipient sites having good blood circulation, the development of large areas of bone was observed around the regenerated cartilage.

Lester (1959) documented the production of cartilage and bone by perichondrium in two patients who had undergone subperichondrial resection of costal cartilage. Experimentally, the chondrogenic potential of perichondrium was first recognised in rabbits by Skoog *et al* (1972). Since then, additional experimental studies (Sohn and Ohlsén, 1974; Engkvist *et al.*, 1975; Ohlsén *et al.*, 1975; Ohlsén and Nordin, 1976; Skoog and Johansson, 1976; Engkvist, 1979; Engkvist and Ohlsén, 1979; Engkvist and Wilander, 1979; Engkvist *et al.*, 1979; Ohlsén and Widenfalk, 1983) and several clinical cases (Brent and Ott, 1978; Tajima *et al.*, 1978; Engkvist and Johansson, 1980) using free perichondrium have been reported. In almost all experimental studies reported, the resulting tissue was described as almost normal cartilage. However, we have often observed the development of bone around the neocartilage in perichondrial grafts.

In this experimental study, the development of bone after perichondrial grafting was investigated using rabbit ear and rib perichondrium.

Materials and methods

Sixty-four white adult female rabbits weighing 2800–3200 gm were used. The ear and rib were used as graft donors. Rabbits were classified into 4 groups:

Group A (N = 16): Free ear perichondrial graft.
Group B (N = 16): Vascularised ear perichondrial graft.
Group C (N = 16): Free rib perichondrial graft.
Group D (N = 16): Vascularised rib perichondrium.

The rabbits were sedated intravenously with sodium pentobarbitone and anaesthetised through inhalation of a mixture of halothane, nitrous oxide and oxygen. Either the ears or the anterior chest were shaved and prepared with povidone-iodine and operations were carried out under strict aseptic conditions. Eight animals in each group were examined individually at 6 and 18 weeks postoperatively. Graft specimens obtained were decalcified and embedded in paraffin. Sections 10 μm thick were prepared and stained with haematoxylin and eosin and examined under a light microscope. Rabbits which died or whose wounds were infected were replaced by new ones.

Group A

An oval graft of perichondrium measuring 3 × 5 cm was outlined on the convex surface of each ear (Fig. 1). The graft was harvested from the proximal part of the ear in order to contain the intact inner, transitional layer of perichondrium. The existence of this layer was confirmed by microscopic evaluation. It is difficult to get such perichondrium from the distal part of the ear where the perichondrium is rather thin. A skin incision was made along the centre line of the ear and the perichondrium was elevated from the underlying cartilage using an elevator. The harvested perichondrium was tubed with the inner transitional layer inside. This procedure was applied to both ears of 16 rabbits.

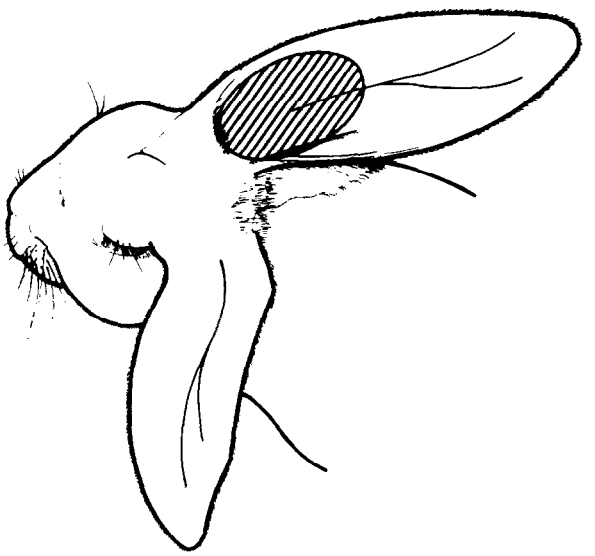

Fig. 1

Figure 1—Donor site of ear perichondrial graft (shaded area).

One of the two pieces of tubed perichondrium was grafted into the liver which was very vascular. The other was grafted into a deep pocket in the abdominal wall at the level of the panniculus carnosus which was expected to be less vascular. All the incisions were then closed.

Group B

A composite free flap, based on the central artery of the ear and the external jugular vein, was elevated. This experimental model of a free flap was reported by Shearin *et al.* (1976). An oval flap measuring 3 × 5 cm was outlined on the same site of the ear as in Group A. This flap included the central artery and the venae comitantes which joined the posterior facial plexus of veins to form the external jugular vein. The incision included the skin and the underlying perichondrium. The perichondrium was elevated with an elevator and then the perichondro-cutaneous flap was harvested. This flap was transferred to a skinned area in the ipsilateral groin region with microvascular anastomosis. The flap was placed with the chondral side facing the abdominal wall. The central artery was sutured end-to-side to the femoral artery and the external jugular vein was sutured end-to-end to the femoral vein. The overlying skin of the flap was checked to monitor the patency of the anastomosed vessels. This procedure was applied to one ear of 16 rabbits.

Group C

The cartilaginous portions of the sixth to ninth ribs were exposed through a transverse incision on the anterior chest. The anterior surface of the perichondrium was incised longitudinally with a blade and about 2 cm of the cartilage was resected subperichondrially from two or three ribs. The perichondrium was then harvested with scissors, care being taken not to damage the pleura. This procedure was applied to both sides of the chest and two or three pieces of perichondrium were harvested on each side. The harvested perichondrium was tubed with the inner transitional layer inside. Two or three pieces of tubed perichondrium were grafted into the liver and the remaining two or three pieces were grafted into deep abdominal pockets.

Group D

The same procedure as in Group C was used to resect two or three costal cartilages on the left side of the chest. The costochondral junctions were kept intact. The remaining perichondrium was tubed *in situ* so that the tubed perichondrium of each rib was nourished by the intercostal vessels. Then all the incisions were closed.

Results

The results for all groups of rabbits are shown in Table 1.

Group A. The incidence of cartilage formation in the abdominal pockets was 75% at both 6 and 18 weeks postoperatively. That in the livers was 88% at 6 weeks and 100% at 18 weeks. The thickness of regenerated cartilage varied from a single row of chondrocytes to a broad band of cartilage even thicker than the original ear cartilage. In the former, the development of small areas of bone could be found around the regenerated cartilage in 2 rabbits (25%) at 6 weeks and 4 rabbits (50%) at 18 weeks (Fig. 2). In the latter, large areas of woven fine cancellous bone were observed simultaneously in every specimen which contained regenerated cartilage. The development of central marrow cavities could also be observed (Fig. 3).

Group B. In every specimen at both 6 and 18 weeks, cartilage formation could be macroscopically ob-

Table 1 Incidence of cartilage and bone formation in each group. Total number in each group: 8 grafts.

Group	At 6 weeks		At 18 weeks	
	Cartilage	*Bone*	*Cartilage*	*Bone*
A Free ear perichondrial graft				
In abdominal wall	6 (75%)	2 (25%)	6 (75%)	4 (50%)
In liver	7 (88%)	7 (88%)	8 (100%)	8 (100%)
B Vascularised ear perichondrial free flap	8 (100%)	8 (100%)	8 (100%)	8 (100%)
C Free rib perichondrial graft				
In abdominal wall	6 (75%)	1 (13%)	7 (88%)	2 (25%)
In liver	6 (75%)	6 (75%)	8 (100%)	8 (100%)
D Vascularised rib perichondrium	8 (100%)	8 (100%)	8 (100%)	8 (100%)

served in the form of a flat sheet (Fig. 4). The sheet of cartilage was slightly thicker than the original ear cartilage. Microscopic evaluation revealed extensive areas of woven fine cancellous bone within cartilage. Some areas of the regenerated bone were continuous with cartilage and appeared to derive from it, while other examples existed without cartilage (Fig. 5). The cartilage at 6 weeks, which consisted of small chondrocytes and faintly stained intercellular substance, was immature. However, that at 18 weeks had become mature and resembled the original ear cartilage.

Group C. The incidence of cartilage formation in the abdominal pockets was 75% at 6 weeks and 88% at 18 weeks. That in the livers was 75% at 6 weeks and 100% at 18 weeks. There were no differences in the volume of graft specimens between the two. In the former, the development of small areas of bone was observed at the peripheral sites of the regenerated cartilage in 1 rabbit (13%) at 6 weeks and 2 rabbits (25%) at 18 weeks (Fig. 6). The cartilage at 6 weeks was of an immature type with rather small chondrocytes and faint staining of the intercellular substance. That at 18 weeks had become mature and resembled original rib cartilage. In the latter, large areas of woven fine cancellous

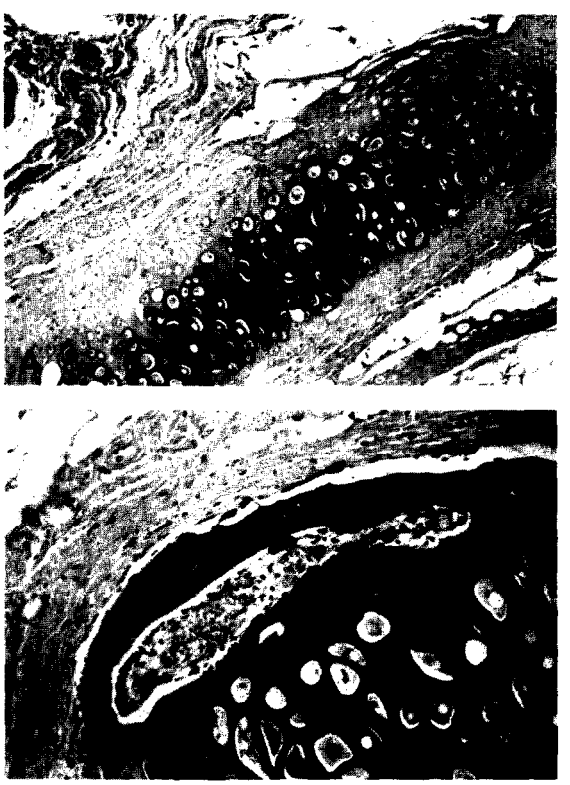

Fig. 2

Figure 2—*Group A.* Free ear perichondrial graft in an abdominal pocket (A) Regenerated cartilage at 6 weeks [H.E. ×24]. (B) Woven bone at periphery of cartilage at 18 weeks [H.E. ×60].

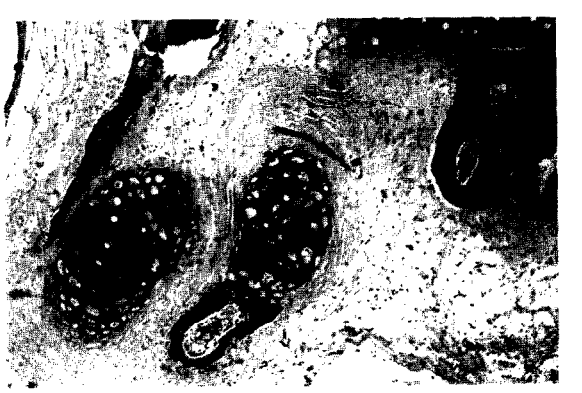

Fig. 3

Figure 3—*Group A.* Free ear perichondrial graft in liver: Regenerated cartilage and woven bone at 6 weeks [H.E. ×12].

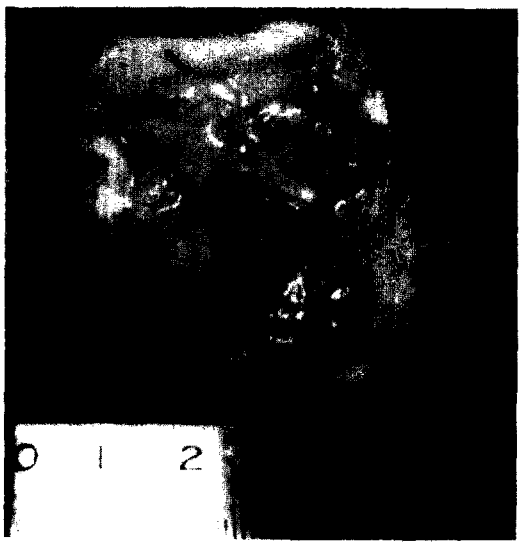

Fig. 4

Figure 4—*Group B.* Vascularised ear perichondrial graft: A sheet of cartilage beneath the skin at 6 weeks.

Fig. 5

Figure 5—*Group B.* Vascularised ear perichondrial graft: Regenerated cartilage and woven bone at 18 weeks [H.E. × 12].

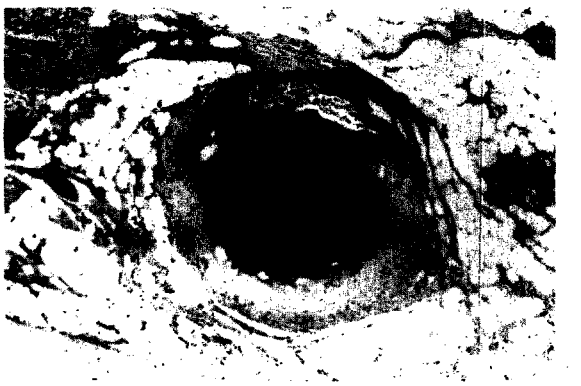

Fig. 6

Figure 6—*Group C.* Free rib perichondrial graft in an abdominal pocket: Woven bone at periphery of cartilage at 18 weeks [H.E. × 12].

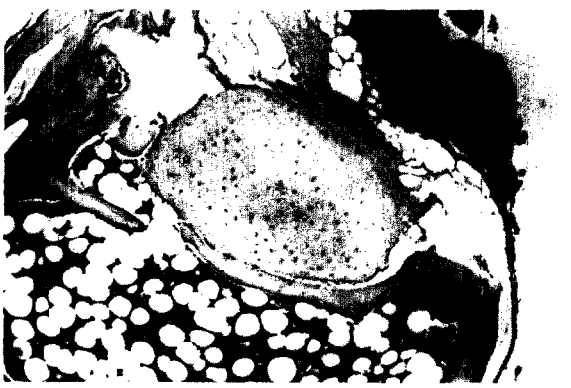

Fig. 7

Figure 7—*Group C.* Free rib perichondrial graft in liver: Regenerated cartilage and woven bone with central marrow cavity at 6 weeks [H.E. × 24].

bone were observed simultaneously in every specimen which contained regenerated cartilage (Fig. 7). The cartilage at 6 weeks had already become as mature as rib cartilage. The development of central marrow cavities was observed in almost all the specimens at both 6 and 18 weeks.

Group D. X-rays at both 6 and 18 weeks showed the evidence of calcification at the sites of the defects of cartilage (Fig. 8). Macroscopically, the defects of rib cartilage were replaced by tissues which resembled bone and which distinctively demarcated the sites of resection (Fig. 9). Microscopic evaluation revealed much fine-fibred compact bone containing large areas of cartilage at both 6 and 18 weeks. The structure of the regenerated bone at 18 weeks resembled that of rib in cross-sections (Fig. 10).

Discussion

In this experimental study, the incidence of cartilage and bone formation in perichondrial grafts and the conditions necessary for their production were investigated using ear and rib perichondrium of rabbits.

The incidence of cartilage formation in our study was 75% or above in every group. Engkvist *et al.* (1979) reported that, both *in vitro* and *in vivo*, rabbit

Fig. 8

Figure 8—*Group D.* Vascularised rib perichondrium: X-ray at 6 weeks. Arrow indicates calcification at the defects.

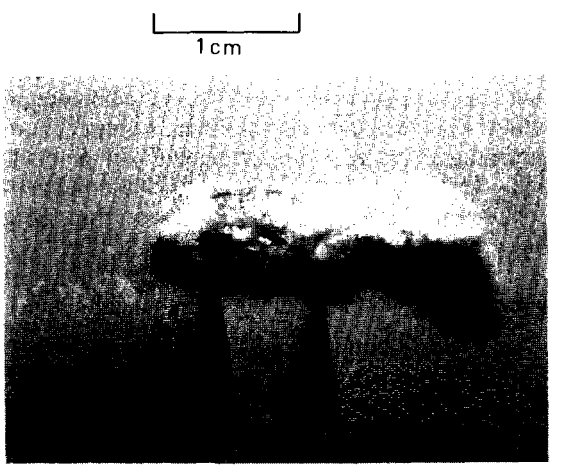

Fig. 9

Figure 9—*Group D.* Specimen at 6 weeks: Regenerated bone-like tissue between the arrows.

rib perichondrium appeared to have greater chondrogenic potential than ear perichondrium. Kon and van den Hooff (1983) were of the same opinion as regards the differences between the two. They suggested that the absence of the inner, transitional layer of perichondrium harvested from the ear cartilage was the reason for such results. However, in our study there were no significant differences between the two kinds of perichondrium used. One explanation is that the perichondrium harvested from the proximal part of the ear could be elevated, preserving the inner, transitional layer.

There was no significant difference between the incidence of cartilage formation in the abdominal pockets and in the liver. The thickness or volume of regenerated cartilage was also about the same. Kon and van den Hooff (1983) reported similar findings in their comparison of intramuscular and subcutaneous insertion of perichondrium of the rabbit. Donski and O'Brien (1980) transplanted

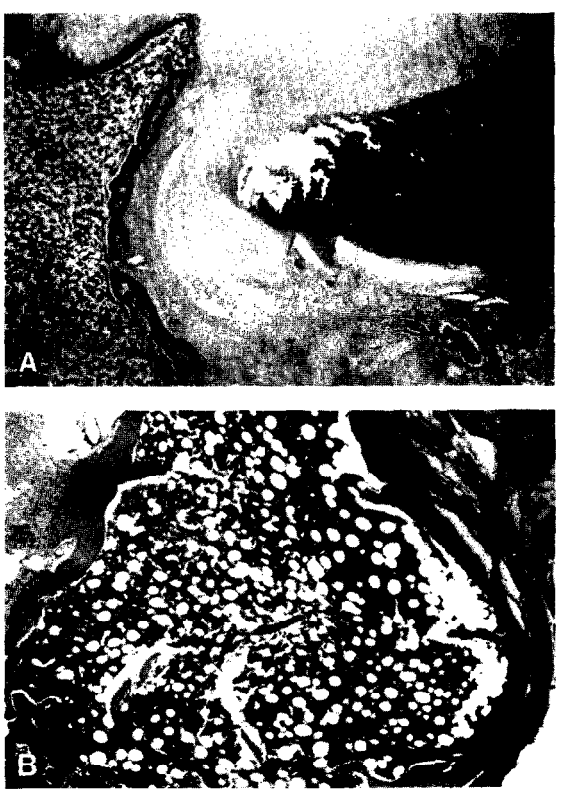

Fig. 10

Figure 10—*Group D.* (A) Longitudinal section: Bone distinctively demarcating the resection site at 18 weeks [H.E. × 12]. (B) Cross-section: Fine-fibred compact bone has central marrow cavity at 18 weeks [H.E. × 12].

free and vascularised grafts from rabbit ears to the lower abdominal walls and compared the formation of cartilage. They stated that vascularisation appeared to have little influence on the cartilage formation from perichondrium. The result was that both free and vascularised perichondrium showed completely mature cartilage cells only 1 month after the operation. Our study confirmed the excellent chondrogenic potential of ear and rib perichondrium whether vascularised or not. It is very interesting that this is contrary to the osteogenic potential of periosteum, which appears to depend mainly on the blood circulation (Takato *et al.*, 1986).

In addition to cartilage, the development of bone was observed simultaneously in every group of our experimental study. Small areas of bone were observed in less than 50% of free perichondrial grafts in the abdominal pockets but large areas were present in almost all cases of free perichondrial grafts in the liver. Furthermore, extensive areas of woven fine cancellous or fine-fibred compact bone were observed within regenerated cartilage in every case of vascularised ear and rib perichondrium and central marrow cavities were also found. These findings suggest that bone developed when perichondrial grafting took place under conditions of good blood circulation but it was not clear whether the bone was formed from the perichondrium itself or secondarily from the cartilage.

The development of bone after perichondrial grafting has only rarely been reported (Kon and van den Hooff, 1983), probably because perichondrium has usually been grafted in subcutaneous tissues or used in the reconstruction of joints which are mainly nourished by synovial fluid. It follows that we should take close notice of the fact that the perichondrial grafts in well vascularised recipient sites can give rise to bone as well as cartilage.

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