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PERICHONDRIAL MICROVASCULAR FREE TRANSFER: AN EXPERIMENTAL STUDY IN RABBITS

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THE regenerative power of perichondrium has been recognised by Skoog and his co-workers (1972) and was investigated in experimental reconstruction of tracheal defects (Sohn and Ohlsen, 1974; Ohlsen and Nordin, 1976) and the formation of articular cartilage (Scoog and Johansson, 1976). Clinically this potential was used in the treatment of the cauliflower ear and to correct protruding ears by periochondroplasty (Skoog, 1974), the reconstruction of small joints (Skoog, 1976; Tajima, 1978) and as perichondro-cutaneous grafts in ear, nose, eyelid and septum reconstruction (Brent and Ott, 1978).

Free isolated perichondrial grafts produce much less cartilage than combined perichondro-cutaneous grafts and seem to need the additional stimulus of blood for cartilage formation (Brent and Ott, 1978). The potential of cartilage formation of microvascular perichondrial grafts was investigated in this experiment to ascertain whether more predictable and possibly more substantial cartilage formation would develop.

MATERIAL AND METHODS

Thirty-five New Zealand white rabbits, 2 to 20 months old and weighing 2-2·5 kg were operated in several groups, consisting of 5 rabbits each. Perichondrial grafts from both ears were transplanted to the lower abdominal wall. Vascular anastomoses were accomplished on 1 side, the other side acting as control. Exploration and sacrifice for macroscopic and histological evaluation were performed after 2 weeks, 1, 2, 3 and 6 months. In addition, grafts at 1 week and fresh biopsies from rabbit ears were studied. In another group the perichondrial grafts were wrapped around a silicone framework, similar to an ear framework. This was discarded after 3 months and the rabbits sacrificed 5 months after the original procedure.

Operative procedure. The animals were anaesthetised with intravenous pentobarbital plus inhalation of Halothane, nitrous oxide and oxygen. An oval graft measuring 4×6 cm in diameter was outlined on the convex surface of each ear. The skin was removed, leaving the underlying vascular structures intact. Along the graft margin the soft tissues were incised, including the perichondrium. The graft was detached from the cartilage by a periosteal elevator or knife, keeping the blade in a sideways position and peeling the perichondrium off by a scraping motion. On 1 side the principal artery and vein of the ear were dissected as a vascular pedicle for the graft (Fig. 1). An incision in the groin was used for access to the epigastric and femoral vessels and to the lower abdominal wall. On 1 side the graft was fixed to the abdominal wall and the epigastric vessels were anastomosed to the flap vessels. On the other side the graft was sutured to the abdominal muscles only without vessel anastomoses. End to end vascular anastomoses were performed under the operating microscope with a 19 μ Nylon on a metallised

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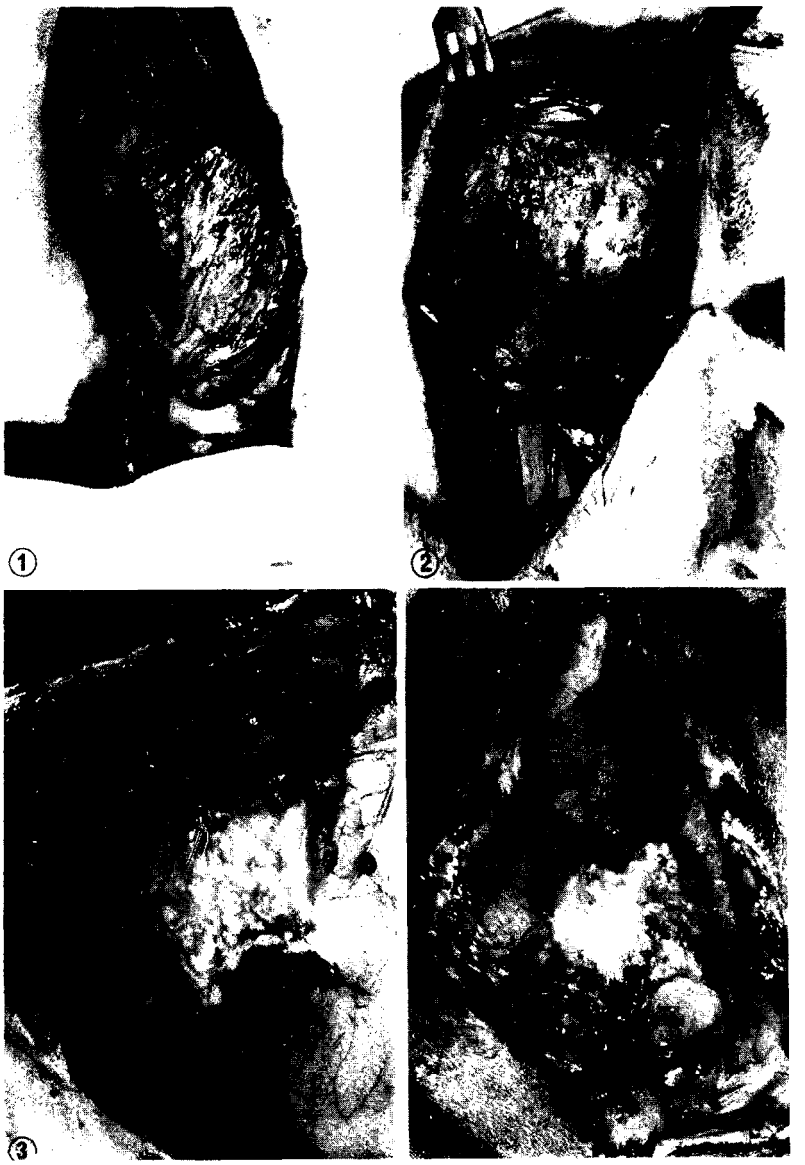

FIG. 1. Dissected perichondrial graft, attached by its vascular pedicle.

FIG. 2. Revascularised perichondrial graft sutured to the lower abdominal wall.

FIG. 3. Revascularised perichondrial graft at 2 weeks. Note the cobblestone-like appearance of the newly formed cartilage.

FIG. 4. Revascularised shrunken perichondrial graft at 5 weeks: a compact plate of cartilage has formed.

needle. The arteries measured 0·9-1·2 mm, the veins 1·0-1·8 mm. Six to 8 silk sutures were used to fix the grafts into place under moderate tension. The undersurface of the perichondrium was directed towards the skin (Fig. 2). Because of considerable discrepancy in the diameter of the graft vein and the smaller epigastric vein, anastomosis of the auricular vein was then performed in end to end fashion to the femoral vein.

RESULTS

Three out of 35 revascularised grafts showed vascular thrombosis at exploration (patency rate 91 per cent). Infection was no problem but in 3 animals remnants of skin appendages caused formation of large cysts (this occurred in vascularised grafts as well as non-vascularised grafts).

Macroscopic findings. Despite individual variation, vascularised and non-vascularised grafts showed similar changes with approximately the same amount of

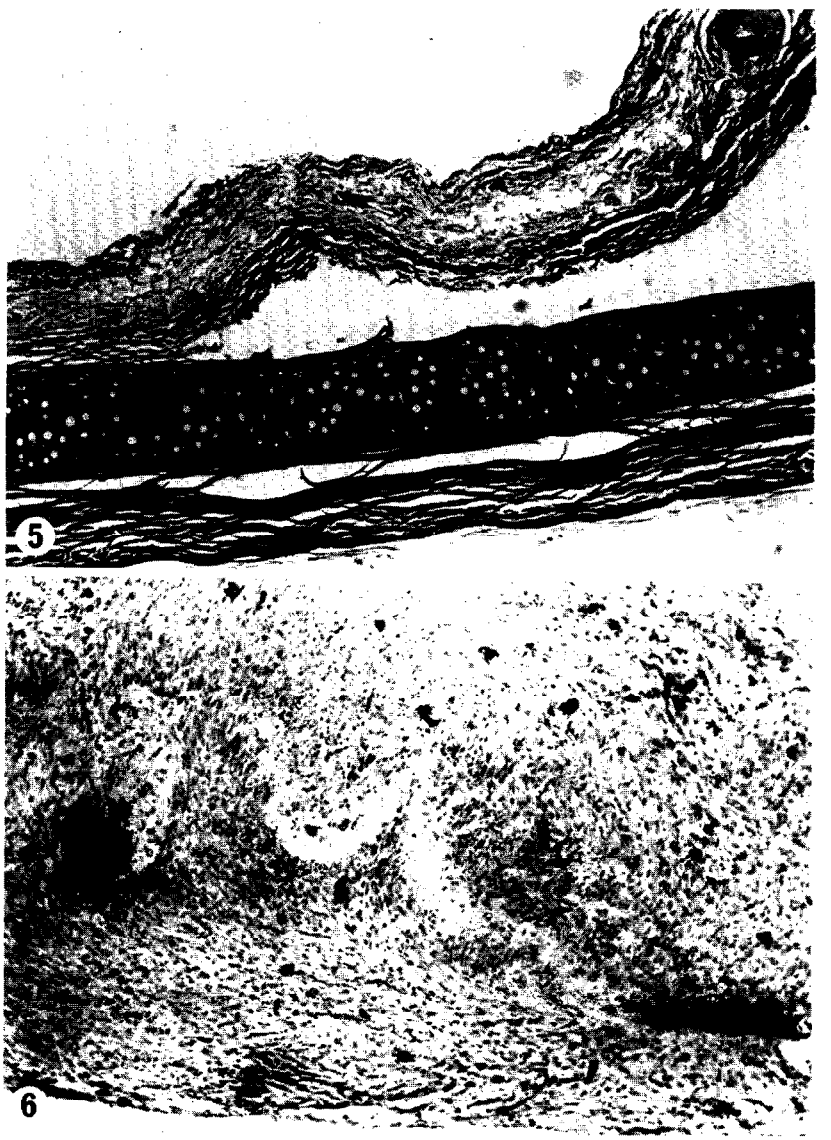

FIG. 5. Histology of perichondrial graft being separated from the ear cartilage.
FIG. 6. Histology at 1 week in a vascularised graft. Description in text.

cartilage formation. The grafts were not thicker than 2-4 mm at the time of sacrifice of the animals. Grafts at 1 week did not show any remarkable macroscopic changes. Cartilage formation was visible at 2 weeks in a patchy cobblestone-like appearance (Fig. 3). At later stages these patches often coalesced and formed a more compact plate (Fig. 4). At the same time considerable shrinkage of the grafts took place to about one-third of the original size. This was possible because of the underlying mobile and elastic abdominal wall. Identical changes occurred in the grafts wrapped around a silicone frame. After removal of the framework these grafts collapsed partially and remained in this position until they were harvested for examination at 5 months.

FIG. 7. Histology at 2 weeks, demonstrating wide band of cartilage formation. A. Vascularised graft; B. Non-vascularised graft.

Histology. Paraffin sections, 10 μ thick, were stained with haematoxylin and eosin for general examination, Aldehyde-Fuchsin-Gomori (AFG) for collagen and elastic fibres and Alcian blue, Periodic Acid-Schiff and Van Gieson (APV) for muco-polysaccharides (ground substance) of cartilage.

Immediate sections of elevated perichondrial grafts showed occasional small, and sometimes more extensive areas of cartilage, 1-2 cells thick attached to the undersurface of the grafts (Fig. 5). In a specimen obtained at *1 week* the vascularised graft showed hyperplastic perichondrium with numerous plump spindle cells merging with surrounding connective tissue. An incomplete ribbon of specifically staining characteristic

FIG. 8. Histology at 1 month. Cartilage formation has increased with mature cartilage cells. New cartilage is still forming on the subperichondrial surface. A. Vascularised; B. Non-vascularised control graft.

intercellular substance had developed, in which numbers of cells with pale staining nuclei and some surrounded with clear spaces were seen. In a few places the cells were more mature, present in groups and resembled cartilage cells. Some very mature pieces on the surface were probably cells stripped away from the ear cartilage (Fig. 6).

The corresponding non-vascularised specimen at 1 week did not show the active proliferation of the other specimen. A number of loosely arranged cells with stellate nuclei surrounded 2 small islands of intercellular substance in which a few single cells with pale staining nuclei were seen. These islands represented the earliest stage of cartilage formation and were neither as mature nor extensive as in the vascularised graft.

Specimens at *2 weeks* appeared similar in vascularised and non-vascularised grafts.

FIG. 9. Histology at 3 months. Description in text. A. Vascularised; B. Non-vascularised control graft.

In some there was an almost continuous wide band of cartilage formation. On the original subperichondrial surface the cartilage cells were immature and matured outward to almost mature cartilage. On this surface occasional fragments of original cartilage stripped from the ear could be identified. The thickness of the newly formed cartilage averaged 2-3 times the original ear cartilage (Fig. 7).

In specimens at *1 month* the vascularised and non-vascularised specimens continued to present a similar picture. Cartilage was thicker than at 2 weeks and showed completely mature cartilage cells. However, some new cartilage appeared to be still forming on the subperichondrial surface (Fig. 8).

At *2 and 3 months* the histological picture was similar: the mature cartilage was now surrounded by a perichondrium of uniform thickness and resembled the mature elastic cartilage of the rabbit's ear. The interstitial substance was compressed into thin bands by the rounded chondrocytes present in large lacunae. The interstitial matrix contained a dense network of elastic fibres (Fig. 9).

No further changes were observed in specimens examined at 6 months.

Discussion

Chondrogenesis results from loss of "contact inhibition" when the perichondrium is stripped from the underlying cartilage as in the formation of the cauliflower ear. Cartilage formation from free perichondrial grafts was investigated by Ohlsen (1976). Perichondrium of the size of 15×15 mm was taken from the rabbit's ear and transplanted to different areas of the body. At randomly selected recipient sites, a clot of blood was placed in close contact with the perichondrium. Wherever the perichondrium was grafted in the presence of clotted blood, the new cartilage was produced irrespective of age and weight of the rabbit. When blood was withheld, little or no cartilage formed. The new formation began in the first week after transplantation and the cartilage was mature within 7 weeks (Ohlsen, 1976). These experiments confirmed the former findings by Skoog, Ohlsen and Sohn (1972) of limited neochondrogenesis in free perichondrial grafts placed in beds without haematomas. Recently composite perichondro-cutaneous grafts were studied by Brent and Ott (1978). Cartilage precursor cells were seen in the flaps at 7 days and differentiated so rapidly that by 14 days their intercellular spaces were noticeably increased and the formation of a distinct cartilaginous plate well advanced. Macroscopically cartilage formation could be seen at 10-14 days in the form of an uneven cobblestone appearance. This changed gradually to a smooth, flat appearance during the following 6 to 10 weeks as maturation progressed. Eighty-six per cent of the healed perichondro-cutaneous grafts formed a uniform cartilaginous sheet, but only in 50 per cent of the free perichondrial grafts was cartilage production detected. The amount was limited strictly to scattered small islands of cartilage, and only in a few instances sheets of cartilage were formed (Brent and Ott, 1978).

In our experiment, cartilage formation varied considerably in different animals. These relatively large grafts of perichondrium presented some problems in their dissection. Adherence of the perichondrium to the auricular cartilage is not the same in all animals and varies, depending on its location within the flap. A ridge of close attachment was regularly encountered in the proximal part of the grafts and at the distal periphery the grafts were very thin. As could be seen in the fresh biopsies, some underlying cartilage was included in small amounts in the grafts. This did not disturb the outcome of the experiment since the same method of dissection was used in vascularised grafts as in the controls. The thickness of cartilage formed was up to 3 times that of the normal ear at 1 month. This is in accordance with findings by Ohlsen (1976) who reported a maximum thickness at 2 weeks (on average 4 times the normal thickness of a

rabbit's ear) that was reduced to no more than 1·5 times that of the rabbit's normal ear at 2 months.

Since cartilage formation was not even, and often not forming a completely uniform plate, the stability of such cartilage is greatly reduced compared with the relatively thin but undisturbed plate of the donor ear. Formation of a continuous sheet of cartilage is therefore dependent on a most careful dissection technique, that avoids any damage to the delicate perichondrial structure.

It was not possible to show any distinct difference in cartilage formation in the vascularised and non-vascularised grafts. Although the findings in 1 week grafts suggested that cartilage formation might be retarded on the non-vascularised side, no difference was apparent at 2 weeks. Since in the groin dissection no attempt at absolute bloodless dissection was made and the non-vascularised grafts contained blood in the graft vessels, cartilage formation occurred in all these grafts in the same way as in free perichondrial grafts in the presence of deposited blood clots.

The absence of any marked differences in cartilage formation in vascularised and non-vascularised grafts is obviously due to the fact that the non-vascularised perichondrial graft is a thin structure that establishes vascular connections in a short time and that the cells survive this stage without harm and preserve their potential. On the other hand, cartilage formation in a vascularised graft cannot exceed a certain amount due to self-limitation of its production.

SUMMARY

Vascularised and non-vascularised perichondrial grafts from the rabbit's ears were transplanted to the lower abdominal wall and their cartilage formation compared. Similar results were found in both groups. Presence of blood and spontaneous revascularisation of non-vascularised grafts as well as self limitation of cartilage formation in immediately revascularised grafts seem to be the reason for these results.

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