Bone Healing after Implantation of Some Hetero- and Alloplastic Materials: An Experimental Study on the Guinea Pig

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The early treatment of children with clefts of the lip and palate has received much space in the literature. Primary bone grafting was introduced by Schmid (9) and Nordin and Johansson (8), and it has been maintained that bone grafting not only prevents maxillary collapse, but also creates the necessary conditions for normal growth of the middle third of the face. But only scanty information is available on the growth of the maxilla following such treatment. Skoog (10) tried the osteogenetic capacity of the periosteum by establishing continuity across the cleft with periosteal flaps from the bordering maxillary segments. He found this procedure to result in the formation of new bone in the alveolar cleft. In one of the cases described, subperiosteal packing with hemostatic sponge was used and was believed to have contributed to the favorable formation of new bone in that case. In a later paper, Skoog (11) used subperiosteal packing with Surgicel and found it to have a very good effect on the formation of new bone. Whether the method with periosteal flaps ("boneless bone grafting") also stimulates normal growth of the maxilla remains to be proved.

In recent investigations, Stenström and Thilander (12) showed that autogenous bone graft from the iliac crest for filling a defect in the premaxillomaxillary suture in guinea pigs, 1 to 3 days old, resulted in a deviation of the nose towards the side of the defect and, hence, in marked asymmetry. Thus, the bone grafting does not seem to stimulate a normal growth of the facial skeleton. In connection with these experiments, which were begun in 1963, experimental studies were also carried

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out on the guinea pig in an attempt to find out whether some hetero- and alloplastic materials might have a favorable effect on bone healing. The results of that study and some clinical observations are reported below.

**Materials**

Ivalon (Ivano Inc.). This is a polymer of polyvinyl alcohol with formaldehyde and for surgical use it is white, porous and, when moist, resilient. Ivalon has been studied in various investigations in recent years and has also been tried clinically (2, 4, 5, 14). Good results have been reported. It is believed not to induce bone formation *per se* (1), but when inserted in contact with spongiosa, bone tissue has been demonstrated in the spaces of the sponge (3, 18). Ivalon was therefore tried in this investigation.

Collagen. Collagen constitutes 35% or more of the organic substance of the bone. It should therefore be of interest to try a material produced from collagen. Two such materials made from the Achilles tendon of cattle were studied.

Collagen Chips (Sigma). Yellow-white, thin dry chips up to the size of drawing pins were used. When moist these chips are resilient.

Band-Shaped Collagen (Ethicon). Greenish bands, about 4 mm wide and 1/2 mm thick. They are commercially available, ready for use, in alcohol in glass ampules.

Surgicel (oxidized regenerated cellulose) (Johnson and Johnson). A cellulose product, which is one of our commonest hemostatics. It is commercially available in sterile ampules in the form of white, shiny silvery gauze-like strips which are easy to apply to a defect. When Surgicel is brought into contact with blood it turns immediately into a dark brown gelatinous mass, which acts as an artificial clot. It is well tolerated and is also resorbed quickly (6, 7). Utilization of these observations was considered useful for our study.

**Operative Technique**

After sedation by intramuscular injection of 0.10 to 0.20 ml of Numal (Roche), anesthesia was induced by infiltration with a tuberculin syringe using 0.20 to 0.25 ml of 0.5 per cent Citanest-Exadrin (Astra). The left side of the guinea pig’s snout was epilated with the use of mosquito forceps, and carefully cleansed. The lower part of the premaxillomaxillary suture was then exposed by means of an incision placed parallel and some millimeters peripheral to the suture. With a dental burr (round no. 5) a defect, 3 x 3 mm, was then made along the exposed suture (Figure 1). Great care was taken not to harm the nasal mucosa and to avoid the development of heat during burring.

The resultant bone defect was either left unfilled (control cases) or filled with Ivalon, Surgicel, or collagen (chips or band-shaped). The
soft parts were then carefully closed, using 5-0 atraumatic catgut subcutaneously and 6-0 atraumatic silk for the skin. In none of the animals was healing complicated.

Since the structures operated on were small, it was essential to wear magnifying spectacles during the operation.

**Microscopic Technique**

In total, 75 animals were used. The guinea pigs were 3 to 7 days old at the time of the operation. After 1 week, 2 weeks, 1 month, 2 months, and 4 months, the animals were decapitated and prepared for histologic examination. Serial sections were cut in the transverse plane, to allow comparison between the operated and intact sides in the same section. The thickness of the sections ranged from 10 to 25 μ. Four successive sections were stained with haemalun-cosin (Mayer), azan (Heidenhain), resorcin fuchsin (Weigert), and picrofuchsin (van Gieson).

**Results**

Healing was never complicated, and in each separate series the results showed a uniform picture.

**Controls.** At the end of the first postoperative week, the defect was filled with granulation tissue and showed signs of incipient regeneration in the periphery; that is, osteoclastic and osteoblastic activity (Figure 2, A and B). After 2 weeks, the osteoblastic activity appeared to be preponderant and bone healing commenced, which continued through the entire series. After 2 months the defect was still open, as it also was after 4 months. An incipient thin bridging was, however, observed. Bone healing continued from the periphery (Figure 2, C and D).

Ivalon filled the cavity within 1 week and was then surrounded by granulation tissue, which penetrated into the spaces of the material.
Figure 2. A, control, 1 week after operation. Signs of regeneration at bone ends bordering the defect. The arrows in this and the following figures indicate the area of the residual defect. B, a higher magnification of the next section in this series. C, control, 4 months after the operation, thin bone plate bridging the defect. D, a higher magnification of Figure C.

(Figure 3, A). Red blood cells were seen and Ivalon appeared to be indifferent to the bony tissues. After 2 weeks the picture was largely the same except that numerous foreign body giant cells could then be seen (Figure 3, B). After 1 month bone healing started in the periphery and continued for up to 4 months, though less rapidly. The defect was still open after 4 months and Ivalon was still to be seen in organized connective tissue, surrounded by eosinophilic areas and foreign body giant cells. Bone healing continued (Figure 3, C, D, E).

Collagen chips filled the defect and were embedded in granulation tissue 1 week after the operation (Figure 4, A). Numerous round cell infiltrates were observed and the chips appeared to be decapsulated from the bony tissue, which in turn appeared inactive (Figure 4, B). The picture was the same after 2 weeks. Collagen chips now began to be attacked here and there in the periphery by macrophages with the results that the margins of the collagen were, so to speak, “nibbled away” (Figure 4, C). After 4 months there were still small islands of collagen which were markedly decapsulated (Figure 4, D). It thus appeared that the collagen chips separated from the bony tissue, which reacted with inactivity, and not until the chips had been resorbed did bone growth commence, which was readily observed after 4 months (Figure 4, E and F).
FIGURE 3. A, the defect with Ivalon, 1 week after the operation. B, numerous foreign body giant cells in the spaces of the Ivalon, 2 weeks after the operation. C, defect with Ivalon, 4 months after the operation. Defect is still open. D, higher magnification of Figure C showing Ivalon in organized connective tissue, surrounded by eosinophilic areas and foreign body giant cells. E, higher magnification of Figure C indicating slow bone healing.

The band-shaped collagen showed largely the same reaction as the chips. After 2 weeks the collagen bands were still unaffected, but macrophages were seen to migrate in toward the ends and to "nibble" at the surface (Figure 5, A). After 1 month this resulted in a fading of the collagen bands (Figure 5, B). After 2 months most of the collagen bands had been resorbed. The bone showed characteristic inactivity lines. In the very periphery there were signs of cellular activity
FIGURE 4. A, defect with collagen chips 1 week after operation. B, higher magnification showing abundant round cell infiltration and inactivity lines in bony tissue. C, collagen chips nibbled away by macrophages, 2 weeks after operation. D, collagen chips, markedly decapsulated, 4 months after operation. E, defect still open 4 months after operation. Residual collagen chips are seen. F, higher magnification showing incipient bone healing.

with osteoblasts and osteoclasts suggesting incipient regeneration (Figure 5, C and D).

Surgicel finally showed quite a different histological picture than the other materials studied. Already within 1 week after the operation there remained only minimal remnants of Surgicel, and the histological picture showed a blood clot with masses of red blood cells filling the defect. The bone began to show definite signs of repair (Figure 6, A, B, C). After 2 weeks, the clot had been replaced by connective tissue. Abundant osteoblasts and marked healing in the periphery were distinct (Figure 7, A, B). The bone growth continued at a good rate and within 2 months all the specimens showed distinct bridging of the defect by bone (Figure 7, C). After 4 months, the defect was filled with bone of practically the same structure as that on the intact side. The bone had, however, not
FIGURE 5. A, band-shaped collagen nibbled peripherally by macrophages, 2 weeks after operation. B, band-shaped rarefied collagen, 1 month after operation. C, defect still open, all band-shaped collagen resorbed, 4 months after operation. D, higher magnification showing incipient bone healing.

quite recovered its original contour but distinct signs suggested that it soon would (Figure 7, D).

Conclusions

The results showed that Surgicel is a material with a very good effect on bone repair and thus appears to be a good substitute for filling different types of bone defects.

Other materials studied did not seem to have such a direct stimulating influence on bone healing; some even had an inactivating effect.

These results therefore induced us to repeat our original experiments on guinea pigs with extirpation of the premaxillomaxillary suture (12) but with implantation of Surgicel in the defects instead of bone grafts. After 3 months the defects were closed, which we interpreted as further evidence of the positive effect of Surgicel on bone healing. In none of the animals was any asymmetry observed in the facial skeleton, in contrast with what was seen after bone grafting.

It is true that the results were admittedly obtained in animals and are therefore not strictly applicable to man. We nevertheless thought that the results were so good that it was justified to implant Surgicel in the cleft of the jaw in 10 children with a cleft jaw and palate, aged about
12 months, in association with soft tissue reconstruction of the cleft of the jaw. We have followed up these children for up to 5 years. Clinically and roentgenologically the results are good. In some cases the defect was spontaneously filled with bony tissue, through which the teeth erupted into the dental arch (Figure 8).

The risk in such cases may, of course, be inhibition of the normal growth of the maxilla, as can be feared in cases with early bone grafting. Growth studies are, however, so far satisfactory. Such studies naturally require a very long observation period for correct evaluation of the late results. It should be remembered that we used Surgicel only in the treatment of clefts of little or moderate width. We do not know whether the same favorable effect can be obtained in the treatment of larger defects. We therefore leave the question open whether Surgicel can produce equally good effects in the treatment of clefts as those once expected of bone grafts but without the disadvantages of the late sequelae of bone grafting. Therefore, we shall not use implantation of Surgicel in the treatment of cleft cases for some years to come.

FIGURE 6. A, minimal remnants of Surgicel, 1 week after operation. B, higher magnification showing abundant red blood cells. C, marked bone healing.
FIGURE 7. A, 2 weeks after implantation of Surgicel, good bone formation peripherally. B, higher magnification of A. C, defect bridged, 2 months after implantation of Surgicel. D, contour of bone almost restored, 4 months after implantation of Surgicel.

FIGURE 8. One case with cleft lip and palate at time of soft-tissue reconstruction and implantation of Surgicel (Nov. 65) in the alveolar cleft and afterwards (May 66, Nov. 66) with spontaneous formation of bone.
Summary

The effect of some hetero- and alloplastic materials—Ivalon, collagen, and Surgicel—on bone healing was studied in the guinea pig. Ivalon and collagen showed no stimulating effect, while Surgicel had a very strong effect. Surgicel therefore seems worth trying as a substitute for filling bone defects in man. Some cases have also been described where the material was used as a filling in the treatment of clefts. No undesired effect of Surgicel has been observed after a follow-up of five years.

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References