THE OSTEOGENIC POTENTIAL OF FREE PERIOSTEAL AUTOGRAFTS IN TIBIAL FRACTURES WITH SEVERE SOFT TISSUE DAMAGE : AN EXPERIMENTAL STUDY

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The present study was undertaken on 80 adult male New Zealand rabbits to assess the effect of free non-vascularized, autologous, periosteum transplants on bone healing in a rabbit fracture model comparable to a tibial fracture with severe soft tissue damage.
Comparison was made between transplantation of free autologous periosteal grafts on the anteromedial side of the tibia and nontransplantation on the contralateral tibia (control). We produced a standardized transverse osteotomy of both tibial diaphyses. The medullary cavity was reamed and nailed ; a 1-cm segment of periosteum was excised from either side of the osteotomy. Periosteal and extraosseous ingrowth was prevented at the osteotomy site by a silastic sheet wrapped around two-thirds of the circumference of the tibia. On the silastic-free bone window, in one group we spanned the osteotomy with a free nonvascularized longitudinally-oriented autologous periosteum sewn to the adjacent periosteum proximally and distally. In the second group, the periosteum was placed transversely with a gap between it and the adjacent periosteum proximally and distally.
Revascularization of the graft was determined with the colored microsphere technique.
Our data suggest that orthotopically-placed autologous nonvascularized periosteum retains its osteogenic potential in a poorly vascularized environment comparable to a tibial fracture with severe soft tissue damage ; the effect is enhanced if the graft is in contact with intact periosteum.
Histologically, callus formation after periosteal grafting resembles endochondral and intramembranous ossification.

Keywords : periosteum ; autograft ; tibia ; soft tissue damage.
Mot-clés : périoste ; autogreffe ; tibia ; lésion des tissus mous.

INTRODUCTION

It has long been recognized that the healing of diaphyseal fractures is related to the severity of damage to the bone and soft tissues at the time of injury (3, 7).

The fracture configuration, presence of infection, blood supply of the fragments and the stability of fixation all have an effect on the rate of fracture union. Gustilo et al. stressed the pernicious influence of periosteal stripping on the healing rate of diaphyseal fractures (6).

The periosteal bone-forming capacity has been well known since the eighteenth century, and was first described by Duhamel de Monceau (4). As far back as 1867, Ollier proved that the deep cellular or osteogenic layer of a free periosteal graft is able to produce bone, and Lacroix demonstrated the osteogenic capability of mature periosteum (9).

Periosteal and osteoperiosteal grafts have been used experimentally in the treament of fractures, loss of bone substance, nonunion, reconstruction of articular defects and femoral neck fractures (1, 19).

There is clear evidence that new bone formation by periosteal grafts may be influenced by the vascularity of the periosteal flap (16), donor site (13), contact with living cortical bone (2, 5),

stress from weight-bearing (13, 5), and the vascularity of the recipient site (15).

The concept of transferring the revascularized periosteum to the diaphysis of long bones to produce weight-bearing bone was presented by Finley *et al.* in 1978 (5).

Van Den Wildenberg *et al.* (19) found in an experiment with the African pygmy goat, that nonvascularized autologous grafts of quiescent tibial periosteum were able to fill a diaphyseal tibial defect with new bone.

Although vascularized periosteal grafts would appear to have all the qualities necessary for bone formation, these microsurgical procedures are long and often arduous.

The aim of the present study was to evaluate the osteogenic potential of longitudinal autologous nonvascularized tibial periosteal grafts in a rabbit trauma model as described by Olusola and Oni (10). The model was modified to simulate a tibial fracture with extensive soft tissue damage, thereby creating a poorly vascularized recipient site.

MATERIALS AND METHODS

The animals were divided into three groups : each animal acted as its own control throughout the experiment. All the surgery was done by the same investigator (P. R.).

1. *Group I* (Fig. 1), 57 adult white male New Zealand rabbits weighing 4.2 to 4.6 kg were used. After intramuscular sedation, analgesia (2 cc xylazine hydrochloride 2% — Rompun®, Bayer AG, Leverkusen), and anesthesia (2.5 cc IM ketamine hydrochloride 50 mg/ml-Ketalar® — Parke-Davis), both tibias were exposed through an anteromedial approach. By extraperiosteal dissection, both tibias were circumferentially freed from the surrounding muscles.

An overall circumferential periosteal defect of 10 mm was created equally proximal and distal to the fracture site. A midshaft transverse osteotomy was performed with a hacksaw. Two-thirds of the bone circumference was covered with silicone sheeting (wall thickness : 2 mm), to prevent revascularization of the underlying cortex via the surrounding soft tissues, simulating a fracture. The osteotomy was stabilized with a specially designed locking nail, 3 mm in diameter (Fig. 2).

A periosteal graft, measuring approximately 2×0.5 cm, was harvested from the proximal metaphyseal region of the contralateral tibia. The graft was transplanted longitudinally on the anteromedial side, between the edges of the silicone sheet, with the cambial layer facing the bone surface. The proximal and distal ends of the graft were sutured to the periosteum lining the fracture, using polyglactin 910-4/0 (Vicryl®). The skin was closed with polyglactin 910-2/0 (Vicryl®).

The control paw on the same animal had a similar operation with omission of the transplanted periosteum. The animals were killed at 4 weeks (n = 6), 6 weeks (n = 13), 15 weeks (n = 12), 19 weeks (n = 10), 28 weeks (n = 10), 40 weeks (n = 1), 49 weeks (n = 1), 54 weeks (n = 1), 62 weeks (n = 1) and 67 weeks (n = 2).

A segment of 2 cm (1 cm proximal and 1 cm distal to the fracture site incorporating the silicone sheet) was cut out of the tibia (Fig. 3).

Each bone segment was radiographically and macroscopically examined. After being embedded in polymethylmethacrylate, three slides of 200-μ thickness were procured from each segment with a sawing microtome and stained with Stevenel's blue/Van Gieson picrofuchsin (14), for histomorphometric analysis of the periosteal callus. The latter was done using enlarged photographs ($\times$ 40) of the histologic sections. Quantification of peripheral callus was done on a calibred transparent grid (15) (Fig.4).

2. *Group II.* Spontaneous revascularization of the periosteal graft was measured in 8 white adult male New Zealand rabbits 1, 2 and 3 weeks after transplantation of nonvascularized longitudinal periosteum. They were sedated and anesthetized as in group I. They were ventilated with a mixture of 40% O_2 and air. Through a midline neck incision, the right carotid artery was exposed and cannulated with a 16-gauge catheter (Desert Medical Inc. Sandy, Utah 84070) ; it was fed to the left ventricle using a pressure transducer as reference.

Five cc (15×10^6) colored polystyrene microspheres (CM) (Dye-Tack Microsphere Trito Technology Inc., San Diego, California U.S.A) was injected slowly (1 min.) into the left ventricle.

A reference sample was collected from a second catheter in the right carotid artery, attached to a withdrawal pump (Harvard apparatus, South Natick, M.A.) at a fixed rate of 10 ml/min for 90 sec.

Blood flows were determined from the ratio of tissue to reference sample activity given by the following formula :

$$\text{Flow (ml/min/100g)} = \frac{\text{tissue activity} \times \text{withdrawal rate} \times 100}{\text{reference activity} \times \text{tissue mass}}$$

Each rabbit was killed by intravenous injection of 15 ml sodium thiopental (Penthotal®, Abbott) 5 minutes after the injection of CM. Both kidneys were harvested and analyzed to evaluate the even distribution of the CM throughout the whole body. The periosteal transplant at the level of the fracture, and a control periosteal flap from the contralateral tibia were taken for histological analysis using hematoxylin — eosin staining.
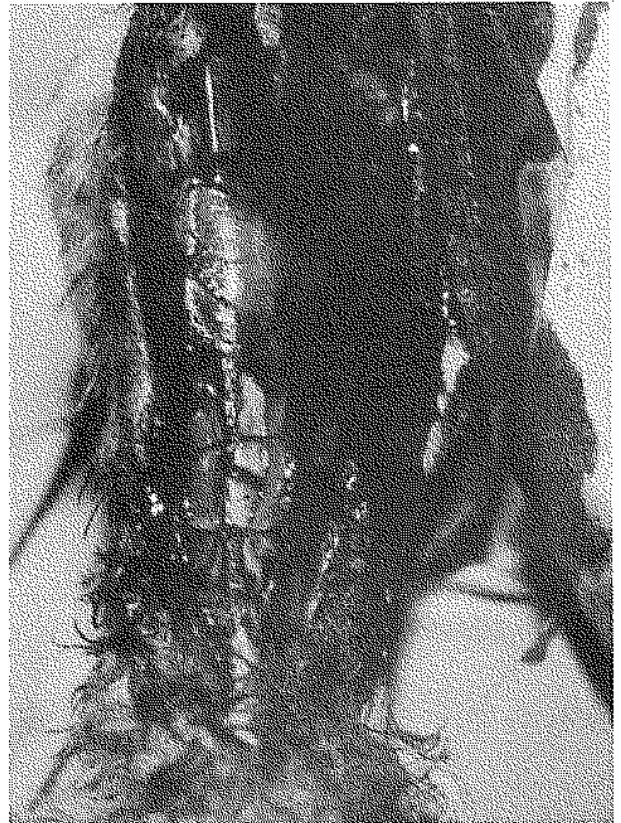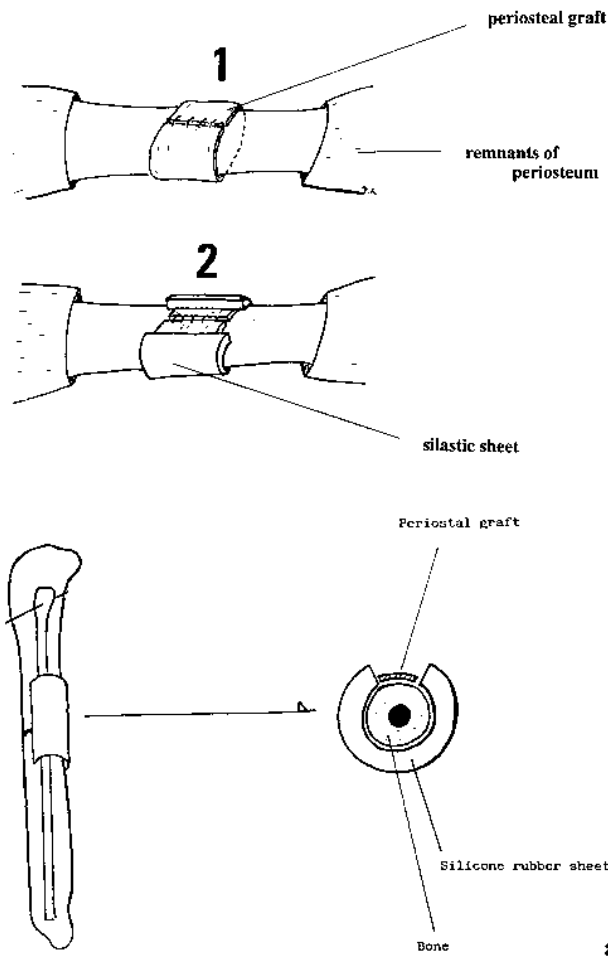
Fig. 1. —
a) Basic draft of the experimental setting in group I.
b) Photograph of a tibia after transverse osteotomy and application of the silastic sheet (large arrow). Proximal nail prominence (small arrow).
c) Photograph of the control paw of the same animal with omission of the transplanted periosteum. Silastic sheet (large arrow head). Proximal nail prominence (large arrow).
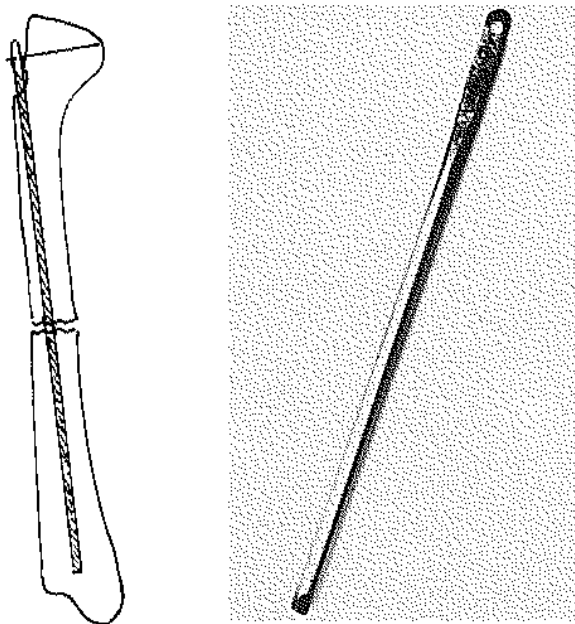
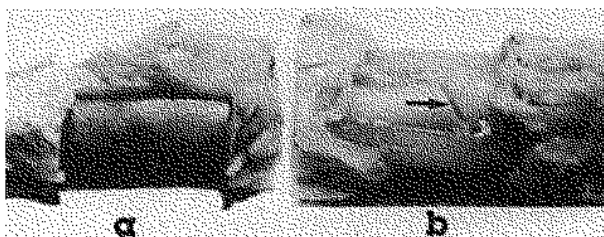Fig. 2.   The intramedullary device used in the experiments.

Fig. 3. — Bone segment cut out of the tibia
a) 20 weeks after periosteal transplantation. The smooth area is the place where the silicone sheet was pressed against the bone.
b) after 20 weeks with no periosteal transplant ; the arrow indicates the fracture line.
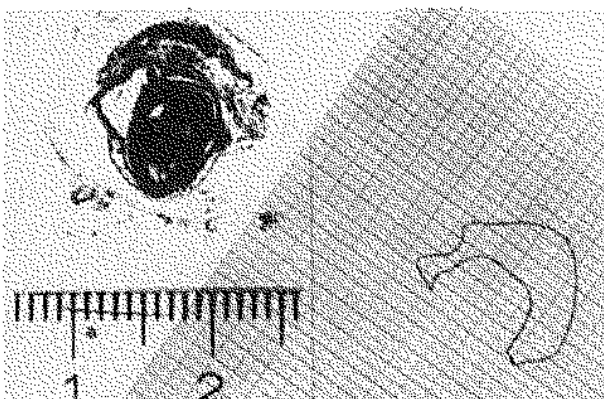

Fig. 4.   Quantification of peripheral callus was done on a transparent grid.

3. *Group III* (Fig. 5). The same procedure as in Group I was used except for the placement of the graft. In 12 adult male New Zealand white rabbits, the graft was placed transversely to the axis of the tibia between the silicone edges and without any contact with the intact periosteum lining the fracture site. At 15 weeks all the animals were sacrificed ; bone segments of both tibias were taken and analyzed as described in group I.
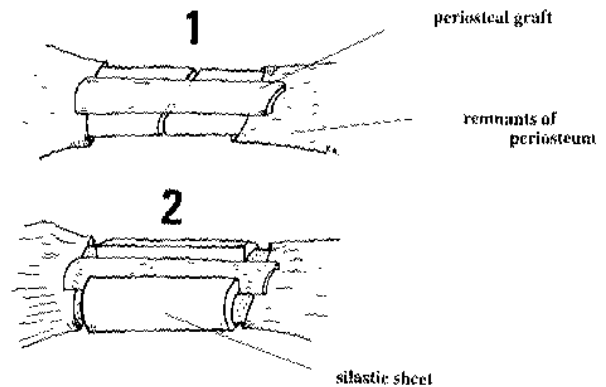

Fig. 5. — Basic draft of the experimental setting in group III.

STATISTICAL ANALYSIS

Callus production was evaluated in two ways : qualitatively as the presence or absence of bridging callus and quantitatively as the callus production on the fracture site.

For the analysis of the qualitative outcome parameter, two statistical techniques were used. Univariate comparison of the presence or absence of bridging callus at each assigned point of time as a function of periosteal grafting was performed with Fischer's Exact test, using $p = 0.05$ as a level of significance.

In the second analysis, for the assigned point of time, the proportion of tibias with bridging callus was calculated, and used as outcome measure. This proportion was then analysed in a bivariate way, as a function of both time and periosteal grafting in a general linear model analysis with partial (type 3) sum of squares. Time was defined as a fixed continuous independent variable. Presence or absence of periosteal grafting was defined as a fixed discrete independent variable.

For the analysis of the quantitave outcome parameter (callus area), the Wilcoxon Signed rank test for paired data was employed.

RESULTS

All rabbits were weight-bearing on the first day after operation. Three rabbits had to be excluded from the study, two due to premature death, and one due to severe osteomyelitis, leaving 77 rabbits for evaluation.

Group 1. Transplantation of longitudinally-placed periosteum (n = 57).

a) The occurrence of bridging callus. We looked for the occurrence of bridging callus (bridging callus versus nonbridging callus) between the tibias with and without periosteal transplantation (table I).

Table I. — Regional blood flow
expressed as mean ± standard deviation

1 week	(n = 2)	0.169 ml/min./gr ± 0.051
2 weeks	(n = 3)	0.111 ml/min./gr ± 0.062
3 weeks	(n = 3)	0.122 ml/min/gr ± 0.110

Up to 6 weeks there was no significant difference in the occurrence of bridging callus between the tibias with and without periosteal transplantation (at 3-4 weeks p = 0.181, at 6 weeks p = 0.073). At 15, 19 and 28 weeks the difference with the controls became significant (15 weeks, p = 0.005 ; 19 weeks, p = 0.010 ; 28 weeks, p = 0.033). At 40 weeks, there were no non-union in the transplant group (n = 6). On the other hand in the non-transplant group there were two non-unions (n = 6), however there was no statistical difference in the occurrence of bridging callus between the tibia with and without periosteal transplantation.

In a comparison between periosteal graft and time, the occurrence of bridging callus in each time frame showed that the variable "periosteal graft" was more significant, (p = 0.005) than time (p = 0.012).

b) Amount of periosteal formation, as measured in the silicone-free window, on the anteromedial side of the tibia.

Up to 4 weeks, histomorphometric analysis of the callus area showed no significant differences between the tibias with and without periosteal graft (p = 0.25). From 6 to 28 weeks we found significantly more periosteal callus in the tibias with periosteal graft, than in those without (6 weeks, p = 0.0002, 15 weeks, p = 0.001, 19 weeks, p = 0.0049 and 28 weeks, p = 0.02). From 40 weeks onwards there were no significant differences in the callus area between experiment and control.

Group II. Analysis of the spontaneous revascularization of the periosteal graft with the colored microsphere technique.

In relation to the blood flow of the contralateral tibia periosteum, the blood flow of the graft is 42.4% ± 7.1 at one week, 92.0% ± 26 at two weeks and 171.5% ± 138.8 at three weeks.

Group III. Transversally placed periosteal graft transplantation (n= 12).

In the longitudinally-placed periosteum transplant group, at fifteen weeks (n = 12), there were 11 rabbits with bridging callus. In the transversally-placed group only 4 of the 12 rabbits had bilateral bridging callus. These differences are significant (p = 0.0021). In these 4 rabbits, calculations of the callus perimeter show no significant differences between the tibias with and without transversally-placed periosteal grafts (paired Wilcoxon signed rank test, p = 0.25).

Histological analysis

Because of the well-defined borders of the periosteal grafts between the silicone edges, periosteal graft procurement with an elevator was very easy. All the grafts in group I and II showed considerable hypertrophy at three weeks (Fig. 6).

Histological sections of the graft at one, two and three weeks are depicted in Fig. 7. Histologically, callus formation after periosteal grafting resembles endochondral and intramembranous ossification.
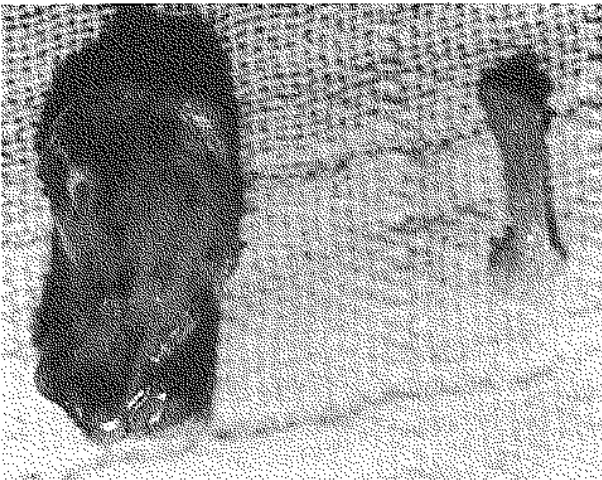
Fig. 6. — Photograph depicting the considerable hypertrophy of the periosteal graft at three weeks ; right : normal appearance of the periosteal graft ; left : situation of the graft three weeks after transplantation.

DISCUSSION

This study was undertaken to investigate the osteogenic potential of a free devascularized autologous periosteum in a poorly vascularized setting, as is often found after trauma. We modified the rabbit tibial fracture model of Olusola and Gregg (10) in order to prevent migration of osteogenic elements from the extraosseous tissue of the calf muscles into the fracture ; the posterior surface of the tibia was covered with a silicone membrane leaving a silicone-free window on the anteromedial surface of the tibia.

Because the periosteal graft is located between the edges of the silicone membrane, it is easy to trace at any time during the experiment. Although bone formation by periosteal grafts is better after microsurgical revascularization (8, 11), microsurgery is a time-consuming and very demanding technique which is not practical for immediate bone covering of open tibial fractures in the presence of severe soft tissue damage. This is the main reason why we chose a practical and reproducible technique of periosteal transplantation which can be used effectively, in the emergency setting, to cover denuded tibial bone after trauma.
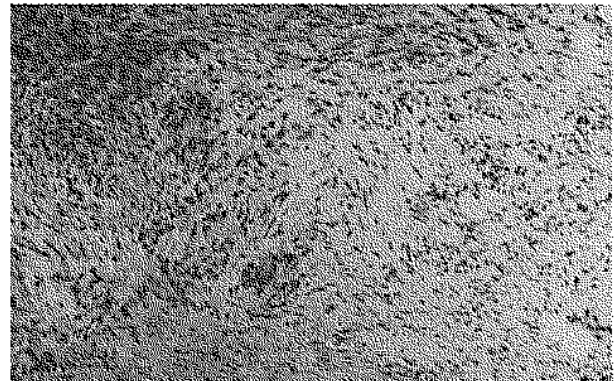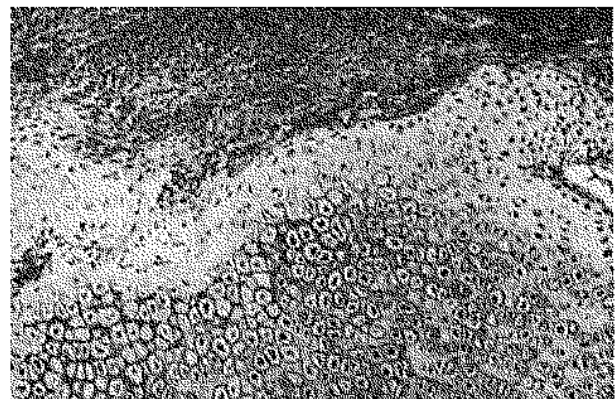
Fig. 7. Histological appearance of a transverse section through a transplanted periosteal graft removed after one, two and three weeks.

a) At one week the transplant contains only young fibroblastic tissue.
Hematoxylin — eosin stain, X 300.

b) At two weeks in addition to connective tissue (**), cartilage is present.
H & E stain, X 300.

c) At three weeks, numerous bone trabeculae are formed, through enchondral ossification (arrow) and intramembranous ossification (large arrow). A small island of cartilage is still present (arrowhead), as well as a rim of connective tissue.
H & E stain, X 120.

In open tibial fractures it is not advisable to cover the fracture circumferentially with periosteum, because surgical dissection at the posterior and posterolateral fracture surface will further damage the remaining muscular connections with the bone, jeopardizing the already critical blood supply to bone. For this reason in the experimental setting, we placed the periosteum only on the anteromedial aspect of the fracture side.

The findings in group I (transplantation of a longitudinally-placed periosteal graft) suggest that transplantation of free autologous tibial periosteum in close contact with the denuded fracture site and the proximal and distal periosteal remnants exhibit statistically significant early bone-forming capabilities even in a poorly vascularized host bed.

The study with the colored microspheres (group II) (21, 22) showed revascularization as early as one week after transplantation. Because of the thickness of the silastic sheet and the location of the test area (silastic-free window on the subcutaneous medial surface of the tibia), there is no contact with the adjacent muscle. This relative isolation of the graft makes it hard to believe that diffusion of a sufficient amount of nutrients from the environment is possible. These results indicate that, in this set-up, the surrounding periosteum will revascularize the graft.

Macroscopic examination showed considerable hypertrophy of the periosteal graft at three weeks posttransplant ; this is not caused by uptake of water but by an astonishing cellular activity of the periosteum. Histological examination showed endochondral and intramembranous ossification as the main pathway to bone healing.

Group III (transplantation of transversely-placed periosteal graft, with no contact with the surrounding periosteal remnants) suggests that the periosteum at the ends of the fracture side is sufficient to revascularize the periosteal graft. When there is no contact with the extraosseous tissue (periosteum and muscle) the graft will quickly resorb.

Although our results are promising, caution should be used in adopting this procedure for clinical use.

Cortical bone may differ greatly from one species to another. In the rabbit and in some hoofed animals, the compact bone consists of layers of lamellar and woven bone with an abundance of blood vessels located mainly in the woven bone (20). In rabbits, bone fragments are bridged by an abundant fibrocartilaginous callus that forms a large collar around the fracture (17). On the other hand, in adult humans cartilage develops at a later stage and appears to be less prominent than in animals (12).

The influence of mechanical stress on the rapidity and quality with which new bone is formed by the periosteum is suggested by several authors (5, 13). Optimal mechanical stimuli in rabbits, like early weight bearing, is a general rule, contrary to humans where fewer mechanical osteogenic stimuli on fractured bones play a role because of splinting, bed rest and delayed weight bearing.

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SAMENVATTING

P. REYNDERS, J. BECKER, P. BROOS. Het primair gebruik van vrije gedevasculariseerde autologe periosteumenten, ter bevordering van de botheling bij tibiaschaftbreuken met ernstige wekedelenschade : experimenteel onderzoek bij het konijn.

De auteur bestudeerde bij tachtig konijnen de osteogenetische eigenschappen van vrije gedevasculariseerde autologe periosteumenten op de beenderige heling in een model dat de toestand van tibiaschaftbreuk met ernstige wekedelenschade nabootst. Het model bestaat uit het creëren van een tibiaschaftbreuk waarna boven en onder de breuk circumferentieel het periosteum over een afstand van 1 cm wordt verwijderd. Ter hoogte van de breuk wordt tweederde van de omtrek bekleed met een siliconen vlies. Het antero-mediaal gelegen testluik wordt bedekt met een periosteument. Aan het controlateraal lidmaat gebeurt een identische ingreep met het weglaten van de periosteument (controle). De periosteumenten werden van de controlaterale tibiametaphyse genomen. Het resultaat (perisotale botaanmaak) werd geanalyseerd door middel van histomorphometrie ; van elke histologische coupe werden vergrotingsopnamen gemaakt, de periostale callus werd gemeten door gebruik te maken van gemillimeterde transparanten. De revascularisatie werd met behulp van de techniek van de gekleurde microsferen bepaald.

De resultaten tonen aan dat spontane revascularisatie van deze greffen mogelijk is, op voorwaarde dat er een innig contact bestaat tussen het transplantaat en de periosteumranden. Zelfs in dit slecht gevasculariseerd gebied blijken deze periosteum transplantaten hun osteogenetisch effect te behouden en versnellen zij aanzienlijk de beenderige heling in vergelijking met de controlezijde.

RÉSUMÉ

P. REYNDERS, J. BECKER, P. BROOS. Le potentiel ostéogénique de greffes libres de périoste dans des fractures tibiales avec dégâts importants des tissus mous : étude expérimentale.

Les auteurs ont cherché à évaluer l'influence de greffes libres, non vascularisées, de périoste autologue sur la consolidation osseuse dans un modèle expérimental chez le lapin, comparable à une fracture tibiale accompagnée de dégâts importants des tissus mous. Ils ont comparé l'évolution de deux groupes de fractures : les unes n'avaient pas reçu de greffe de périoste (contrôles) tandis que les autres avaient reçu des greffes libres de périoste autologue sur la surface antéro-médiale du tibia. La revascularisation des greffes a été évaluée par une technique utilisant des microsphères colorées. Le cal périosté a fait l'objet d'une analyse histomorphométrique, en étudiant avec une grille transparente des agrandissements photographiques des coupes histologiques. Des greffes libres, non vascularisées, de périoste autologue, placées longitudinalement, c'est-à-dire en

contact avec le périoste intact, ont produit significativement plus de cal périosté que les contrôles sans greffe de périoste. Par contre, aucune différence dans la production de cal n'a été notée entre les témoins et les sites fracturaires qui avaient reçu des greffes périostées, placées transversalement dans la fenêtre d'une gaîne de silastic entourant les deux tiers du périmètre osseux. La revascularisation des greffes, évaluée par la technique des microsphères colorées, a été observée endéans la semaine après transplantation. La consolidation osseuse se faisait surtout par ossification endochondrale et membraneuse.

En conclusion, ces observations suggèrent que des greffes périostées autologues non vascularisées en position orthotopique conservent leur potentiel ostéogénique dans un environnement mal vascularisé, comparable à une fracture tibiale accompagnée de gros dégâts des tissus mous ; l'effet est plus important si la greffe est en contact avec du périoste intact. Histologiquement, la formation de cal après greffe de périoste ressemble à l'ossification endochondrale et membraneuse.