Bone Induction Effect of Fine Bone Shavings in Polyester Fibre
AN EXPERIMENTAL STUDY
L. M. JONCK

SUMMARY

Autogenous marrow-free bone shavings of dense lamellar bone spread over polyester mesh were transplanted into muscle, the palate and the tibia. A thin sheet of silastic was used to eliminate the induction effect of the adjacent bone, and the results were evaluated histologically. It was found that finely divided autogenous bone shavings actively induce new bone formation, and that the rate of bone production depends on the proximity of bone-competent tissue, such as a muscle tendon or a muscle attachment. Osteogenesis is also stimulated by a direct blood supply from the adjacent bone.

Polyester mesh (Mersilene) is a relatively inert material, which could be used to great advantage in the transplantation of bone. Preliminary results of this research indicate that it may be of great value in bone reconstruction.

These experiments support the idea of a non-cellular and diffusible bone stimulatory substance which influences the inducible osteogenic precursor cells to produce bone.


The purpose of this investigation was to determine whether or not finely divided bone shavings, collected from compact lamellar bone free of marrow cells, will induce bone formation when implanted into responding tissue.

During the past decade, considerable experimental work has been directed toward studies of the osteogenic potential of the autogenous transplant, implant, and transplant-implantation system. Although many theories have been advanced, the phenomenon of bone induction is still not completely understood.

The present controversy over the merits and demerits of decalcified homogenous bone matrix as a substitute for an autogenous graft began in the late 19th century. Although a 90% rate of success has been claimed, largely with microfilter chambers inserted in muscles, this method has not generally been accepted as a practical surgical procedure. The basic aspect of the controversy is the degree of active osteogenesis by the living cells of an autogenic bone transplant, and the inductive capabilities of the non-cellular organic matrix of the bone itself. Experimental research has indicated that the bone-inducing quality BMP (bone morphogenic protein) is probably harboured in, and is an essential component of, the collagen fibre of the organic portion of the decalcified matrix, and that calcified structures may initially inhibit or even retard bone formation.

In a critical review of this nature, we must also consider the origin of the cell responsible for osteogenesis. From the work of De Bruyn and Kabisch, it appears that the transplant is only responsible for the transformation of mesenchymal cells into osteogenic elements. According to the work of Friedenstein et al., osteogenesis depends upon the osteogenetic precursor cells, i.e. a determined cell (DOPC), which is already committed to osteogenesis, and which is derived from the bone marrow; and the inducible cell (IOPC), which constitutes an auxiliary cell reserve which will only form bone when induced by a specific inductive stimulus, supporting the concept of a blood-borne origin of osteoblasts.

In this investigation, an attempt was made to develop a transplant-implant system in which the optimal osteo-
genesis is effected with a minimal available autogenous bone component. A further requirement is that this method must also be clinically feasible in the restoration of large bony defects.

**MATERIAL AND METHODS**

Primates (8 baboons and 20 monkeys) were used in this investigation. All bone transplants were autogenous bone shavings of compact lamellar bone washed free of marrow cells.

The bone shavings produced by a No. 6 Rosehead tungsten carbide bur were collected by suction under strict sterile surgical conditions in physiological saline in a sterile chamber, and then centrifuged (Fig. 1). The operating sites included muscles, palate, mandible and tibia. Thin silastic sheet was used to separate the implant from the underlying bone. In this way the role of the periosteum and the direct influence of the underlying bone could be clinically evaluated.

Where a double layer of bone was cultivated in the palate, the thin silastic sheet was inserted between two layers of mesh covered with bone particles. A polyester fibre mesh (Mersilene) was used as a base, on to which the fine bone particles were spread.

Of the long bones, the tibia was selected for experimentation, since clinically it is the bone most subject to osteomyelitis, and from an experimental point of view it was more accessible. Large bony defects were created in the tibia by the removal of up to 75% of the bone shaft supraperiosteally. A cylinder of silastic was cut to a length to fill the gap, Kirschner wire being inserted through the centre of the silastic for firmness. The washed bone particles were then spread on to the polyester fibre mesh, which was wrapped firmly around the cylinder of silastic and tied with fine cardiovascular thread (Fig. 2). The transplant-implant was then fitted into the bone defect.

Similar transplant-implants were also inserted directly under a muscular attachment. The influence of a direct blood supply from the bone was determined by the envelopment of the exposed bone in a thin layer of silastic, which would then prevent a direct blood supply from the bone to reaching the transplant-implant; this was inserted under the periosteum (Fig. 3).

*Fig. 1. Suspension of bone shavings in physiological saline in a sterile chamber.*

*Fig. 2. Bone paste spread over the polyester fibre mesh ready for transplantation.*

The surgical procedures were performed under general anaesthesia with naso-endotracheal intubation. Histological specimens were removed at regular intervals for examination.

**RESULTS**

During the first few days, the inter-fibre spaces and the bone shavings were enveloped in an eosinophilic coagulum,
in which red blood corpuscles, a few polymorphonuclear cells, lymphocytes, histiocytes and giant cells could be seen. The whole transplant was surrounded by a typical inflammatory exudate. The giant cells were particularly noticeable in the muscular implants.

After 3 days, a few spindle-shaped cells with deeply-stained nuclei were observed adjacent to the bone shavings and surrounding the polyester mesh. These cells also extended into the eosinophilic coagulum within the mesh. After 5-6 days, most of the implant was filled with vascularised, immature cellular tissue. The majority of these cells were spindle-shaped and contained a moderately chromatic nucleus with basophilic cytoplasm (Fig. 4).

Some of the cells which lay in juxtaposition to the bone shavings were large, and possessed chromatic nuclei with basophilic cytoplasm. The majority of these cells were taken to be osteoblasts. With the formation of an ossicle, the osteoblasts became flattened, each having a long, narrow hyperchromatic nucleus, which appeared to be stretched over the surface of the bone matrix. Protoplasmic processes also extended into the osteoid tissue (Fig. 5).

Within 2-3 weeks, a moderate amount of new bone formation was seen. A few small round cells with hyperchromatic nuclei were present around the capillaries supplying the bone foci. A marked difference was noted where the implant was separated from the underlying palatal bone by silastic. Even after 4 weeks, a relative state of inactivity was noted in the areas where the bone shavings were present, and it appeared that most of the shavings were encapsulated by fibrous tissue. In the fibrous tissue which enveloped the silastic and separated it from the palatal bone, no osteogenesis was detected after 6 weeks, although many of the cells in this layer of fibrinous tissue had the appearance of osteoblasts. However, where the bone shavings were in direct contact with the palatal bone, active bone proliferation took place. The polyester fibres were tightly enveloped by a fibrous tissue with areas of woven bone closely related to it.
The impression gained from the stained sections was that decalcification of bone particles was limited to the surface of the transplanted bone, and that decalcification took place at a very early stage, or even during the process of matrix formation (Fig. 6). The outstanding, and perhaps the most interesting feature, is the sudden appearance of bone mineralisation. The fine bone particles then become surrounded by new bone. It was also found that the bone formation under periosteum was relatively slow when the adjacent bone was covered with silastic. A further interesting feature was the cellular reaction and vascularity outside the periosteum (Fig. 7). Active bone proliferation took place where the bone particles were in direct contact with the adjacent bone. An area of hypermineralisation was noted directly adjacent to the transplanted bone particles.

The thickness of the implant has a bearing on osteogenesis. Where the bone implant was placed directly under the muscular insertion, a dense cellular infiltration around the bone particles was noted within 5 days. Five millimeters deeper, the implant showed less cellular reaction. The whole implant was surrounded with a fibrous layer, which had the appearance of a periosteum. In the control group, where the polyester fibre was inserted without bone particles under the periosteum, in direct contact with the bone, a cellular reaction which indicated osteogenesis was noted, but it was far smaller than when bone particles were included. No bone formation was noted in the control group where the implants without bone particles were inserted in muscle.

In the long bone experiments, where part of the tibia was excised and replaced by the transplant-implant to determine the practical application of the technique, it was found that radiologically new bone formation could be demonstrated within 3 weeks (Figs 8 - 10).

**DISCUSSION AND CONCLUSION**

In this experiment, an attempt was made to employ the general theory of cellular induction. Fine bone particles were used to provide the inducing principle, and although the physicochemical composition of the bone induction principle is not known, experimental results do indicate that the bone induction factor is an essential component of the organic portion of bone. In addition, it is believed that the process of mineralisation also depends upon the physical arrangement of the collagen and molecules in the
basic organic component of bone. From this it can be postulated that the cellular element, derived from the bone marrow, is not necessary to stimulate osteogenesis, and that bone deposition can be effected by the direct induction influence of the bone-inducing factor present in the organic portion of the bone.

These basic principles, the fact that the major part of any autogenous graft is subject to necrosis (especially in those areas not having access to the direct blood supply) and that it will be only the superficial parts which will survive and proliferate, made it reasonable to surmise that fine bone shavings will be more acceptable as a transplant. In these experiments, where the transplant consisted of very fine autogenous bone shavings (12-25 μm thick), evenly distributed over a wide area, and in direct contact with the competent osteogenic tissue and vascular bed, no signs of necrosis were detectable.

From these experiments, the impression was gained that osteogenesis appears to be accelerated by the presence of a direct vascular supply from the adjacent bone. A specific blood supply derived from the adjacent bone may quite possibly contribute additional bone induction factor to the transplanted bone, and in this way it may be responsible for the maintenance of osteogenesis. These principles are well demonstrated by the relative inactivity of the bone transplant in the belly of the muscle and in the palate, where the transplant was separated from the bone by silastic, as compared with the activity of the transplant directly in contact with bone or in the tendon of the masseter muscle.

An inverse relationship between the extent of mineralisation of the implant and the resulting osteogenesis was established by Urist et al. in 1969. The contrary was the case in our experiments, where the sizes of the bone particles were such that they were all in direct contact with a responsive tissue and nutrition. Decalcification and osteogenesis took place simultaneously.

Where the micro-environment was ideal, the process of osteogenesis started immediately, and bone formation was noted within days. The role of the periosteum in osteogenesis is questionable. Mesenchymal cells, once differentiated into the various predestined specialised states, did not respond to the stimulus of the implanted bone particles, and did not produce bone by differentiation into osteocytes. This indicates lack of osteogenic competence.

Apart from the induction effect of the fine bone particles, they may also provide a surface nidus for the deposition of new bone. The slow demineralisation of the bone particles allows enough time for the vascular tissue, which surrounds and envelops the graft, to become induced by the BMP to form osteogenic mesenchyme. There is a critical time period before this happens, in other words, it must be in contact with the responding tissue for at least a certain period of time. The above findings do not support the view of Urist et al. that there is an inverse relationship between the extent of mineralisation of the implant and the resulting osteogenesis. These experiments support the idea of a non-cellular and diffusible bone-stimulating response, which will influence the inducible osteogenic precursor cell to produce bone. The advantages pertaining to this method are:
(i) the greater volume of available bone material due to the small size of the particles;
(ii) the greater surface area of the particles ensuring sufficient nutrition;
(iii) the larger surface area provides greater contact with adjacent tissue and therefore an increased stimulus for bone induction;
(iv) an easier manipulation due to the pliability of the bone paste, allowing better adaptation to the transplant material;
(v) the greater accessibility of donor bone in the immediate vicinity of the operative area;
(vi) more accurately predicted results due to minimal tissue reaction;
(vii) the simplicity of technique places this procedure within the scope of any operator.

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REFERENCES

Books Received: Boeke Ontvang


