Bone regeneration in calvarial defects

Bone defects requiring enhancement of bone healing can be found in numerous circumstances in craniofacial surgery and orthopedics and are often associated with severe functional and esthetic problems

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Bone defects requiring stimulation of bone regeneration can be found as a result of trauma, congenital anomalies, neoplasms, iatrogenic, and infectious conditions. Such bone defects are often associated with severe functional and esthetic problems, and corrective treatment may be complicated by limitations in tissue adaptations and functional liabilities.

Guided bone regeneration is achieved as a controlled stimulation of new bone formation in a bony defect, either by osteogenesis, osteoinduction, or osteoconduction, in order to reestablish both structural and functional integrity. Several procedures and substances have been and are currently used to stimulate bone regeneration in osseous defects in the craniofacial complex.

Clarification of the potential role that growth factors and cytokines play in a variety of tissues and cells and their influence in physiological functions and disease states have expanded in recent years. Treatment applications have consequently been envisaged and have begun to be tested in animal experiments. The accumulation of new knowledge about the multiple regulatory mechanisms involved in bone regeneration will be of prime relevance in understanding physiological and pathological conditions where bone is involved. This is an intent to devise new therapeutic strategies to enhance bone regeneration in calvarial defects based on these novel molecular and biochemical regulatory mechanisms and by means of nonresorbable membranes.

Overview and aims of the experiments

The experimental work of the Ph.D. thesis comprised the following parts 1) development of a comprehensive model to study bone regeneration in rodents, 2) testing whether the experimental model fulfilled the imperative requisites of a critical size bone defect, 3) histological and/or histomorphometric evaluation of bone regeneration achieved by means of polytetrafluoroethylene membranes (PTFE) in rats, and application of a single dose of growth factors, such as transforming growth factor β1 (TGF-β1) and transforming growth factor β2 (TGF-β2) in rats and in baboons.

Development of an animal model in rodents to study bone regeneration

The animal model was developed to evaluate bone regeneration in defects in the craniofacial complex in rats and proved to be appropriate. The advantage of this model in membranous bone is that it does not require bone fixation to stabilize the bone defect, bone regeneration is not affected by mobility nor by muscular activity, does not affect the animal's normal physiological and deambulatory behavior, two equal contralateral bone defects can be produced without involving the sagittal suture and therefore a paired design model is facilitated, and economy of the model with minimal morbidity and mortality. The calvarium due to its relatively biological inertness remains the principal site for the analysis of the potential benefits of bone enhancement in osseous defects.

Critical size bone defect in rats

A critical size bone defect is an osseous defect of a significant size which does not heal spontaneously with bone when produced in adult animals, unless some osteogenic, osteoconductive, or osteoinductive material is placed in or onto it. Frequently, surgically created wounds heal spontaneously, especially in low-order phylogenetic species. For this reason, it is necessary for the control defect to be large enough to avoid spontaneous bone regeneration. Spontaneous bone closure did not occur six and 12 months postsurgery in trephined-produced 5 mm calvarial bone defect in non-growing rats. A 5 mm parietal bone defects in non-growing rats was determined to fulfil the requisites of a critical size bone defect.

Evaluation of bone regeneration

Bone regeneration in calvarial bone defects in rats was evaluated in the following contexts: 1) after placement of a single exocranial PTFE membrane, 2) after simultaneous placement of an exocranial and an endocranial PTFE membrane, and 3)

after a single application of TGF-β1 and TGF-β2 using a gelatin sponge as carrier. A separate experiment aimed to test the effects on bone regeneration of a single application of transforming growth factor β1 (TGF-β1) in insoluble collagenous bone matrix carrier in non-human primates (baboons).

Bone regeneration in calvarial defects using polytetrafluoroethylene membranes

Bone regeneration was achieved in a predictable manner by applying the principles of guided bone regeneration in the calvaria. The placement of an endocranial and an exocranial expanded PTFE membrane placed under the periosteum and over the dura mater to protect the bone edges of the parietal defect from the overlying muscles and underlying brain resulted in clinically significant bone regeneration. However, bone regeneration was not so predictably achieved when a single exocranial membrane was used. The osteogenic role attributed to the dura mater may not be of critical importance in new bone regeneration in calvarial bone defect, and rather may serve as a passive scaffold where bone regeneration can take place.

Effects of a single application of TGF-β1 or -β2 on bone regeneration in calvarial defects in rats

A single application of a low dose of human recombinant TGF-β1 (2, 5, or 10 μg) or TGF-β2 (2 μg) using a gelatine carrier did not promote clinically relevant bone regeneration in membranous calvarial bone defects in adult rats one month post-surgery. A linear-dose related effect of human recombinant TGF-β1 on the histomorphometric percentage of new bone, osteoid, and soft tissue and in the percentage of closure in the experimental calvarial defects could not be demonstrated.

Effects of a single application of TGF-β1 or -β2 on bone regeneration in calvarial defects in non-human primates

A single dose of 5, 30 or 100 μg of TGF-β1 administered in an insoluble collagenous bone matrix carrier had not therapeutical implications for the healing of large cranial bone defects (25 mm) in adult non-human primates one month post-surgery. Islands of cartilage and endochondral ossification were found in the TGF-β1 treated defects, specially in the calvarial defects treated with 100 μg of TGF-β1.

As a conclusion, the placement of a double membrane resulted in clinically significant bone regeneration in calvarial defects in rats, while a single application of TGF-β1 or -β2 did not enhance clinically relevant bone regeneration, neither in rodents nor in non-human primates.

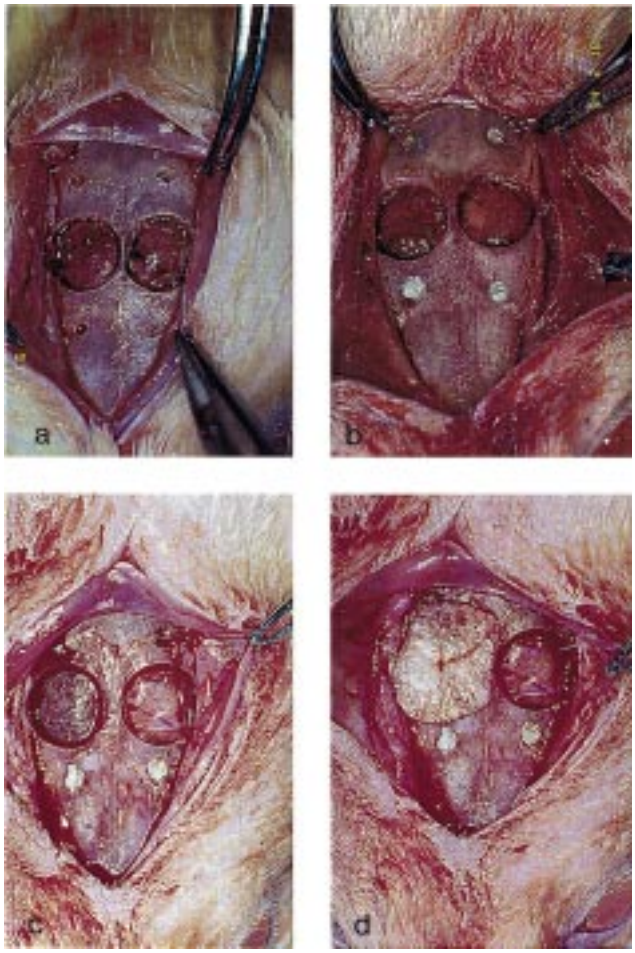

Fig. 1. View of the animal model developed to study bone regeneration in calvarial defects in rats. a: Five-mm calvarial bone defects trephined in the parietal bone. The intact dura mater can be appreciated. b: Amalgam bone markers situated mesially and distally to bone defects to easily identify the center of the defect after bone regeneration. c: The endocranial polytetrafluoroethylene membrane has been placed between the dura mater and the parietal bone in the right defect. d: The exocranial polytetrafluoroethylene membrane has been placed on the top of the defect. The periosteum remains to be sutured on the membrane.

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The thesis was defended at the Royal Dental College, Aarhus University, on November 29, 1996. The opponents were: Professor *Birte Melsen*, DDS, Dr.Odont., and Dr. *Ole Kirkeby*, MD, Odont. Dr.

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