

BONE REGENERATION IN CRITICAL-SIZE CALVARIAL DEFECT IN RATS USING INNOVATIVE NANO COMPOSITE MATERIAL OF CHITOSAN/NANO-HYDROXYAPATITE COMPOSITE

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ABSTRACT

Healing of standardized critical-size calvarial defects in rats was performed with nano composite material of nano-hydroxyapatite/3 % solution of chitosan in citric acid, and electrospinning fibres of poly lactic acid. Histologically at the 84 day, the implanted rats exhibited full closure of the defect by new developed connective and osteoid tissues penetrating in reticular pattern within the implant components. These results indicate that by using a newly created composite paste critical cranial defects can be successfully retrieved and probably is also suitable for other cases in bone augmentation surgeries.

Key words: calvarial defect in rats, chitosan/nano-hydroxyapatite, bone regeneration.

Introduction

Reconstruction of bone defects caused by severe trauma, removal of tumors or congenital malformations still remains a challenge for surgical practice. Therefore, many scientists use different approaches to establish useful bone substitutes for tissue engineering of critical bone defects providing regeneration of bone with mechanical properties similar to the original one. Recently, tissue engineering has become a promising approach in managing bone loss using tissue scaffolds. Tissue scaffolds are the most important issue in tissue engineering and could be divided into two main categories including biological (physical or organic) and synthetic (artificial) materials. Natural polymers such as collagen type I or demineralized bone matrix belong to the first type, while porous metals, bioactive glasses, polylactic acid (PLA) and polyglycolic acid (PGA), and calcium phosphate ceramics such as hydroxyapatite (HA) and tricalcium phosphates (TCP) are examples of synthetic materials [1, 5]. In recent years, particularly useful and reliable as bone substitutes were established some innovative nano-composite materials [6, 9]. Therefore, the aim of the present study was to perform a histological evaluation on the healing potential of a new developed artificial bone graft based on a chitosan/nano-hydroxyapatite composite complex in a standardized critical calvarial bone defect model in rat.

Experimental model.

Eight male rats (Wistar) with critical calvarial defects were divided into two groups with four animals each. Calvarial defects of the rats from group 1 remained untreated, while those in group 2

were filled with composite paste containing nano-hydroxyapatite/3 % solution of chitosan in citric acid, and electrospinning fibres of polylactic acid (NHACHCAPLLA).

Surgical procedure.

All animals were anesthetized by intramuscular injection of xylazine (6 mg/kg) and ketamine (70 mg/kg). After aseptic preparation of the surgical field, in the right parietal zone was made crescentic incision of the skin providing access to the right parietal bone. Using an appropriate dental bur at 2 mm distance from the median line was made parallel critical calvarial defect full bone thickness starting from bone periosteum up to the dura mater, 1,8 mm wide and 6 mm long (Fig. 1a). The defects of the animals from group 2 were filled with nanocomposite material (Fig. 1b), while those in group 2 remained unfilled. The skin paws were adapted over the defect and sutured (Fig. 1c). On day 84 all animals were euthanized with an overdose of pentobarbital and cranial defects were subjected to histological processing.

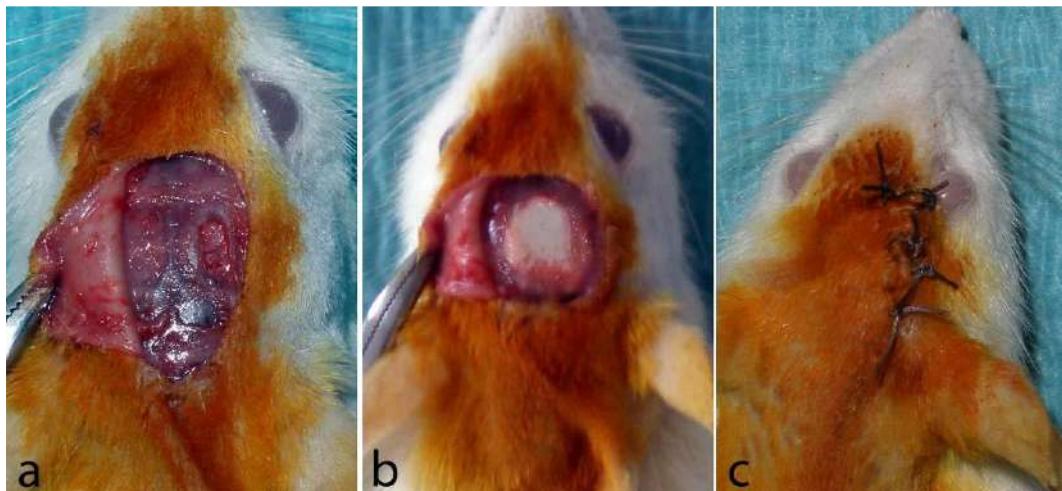


Figure 1: Standardized critical calvarial defect in rat (a) filled with nanocomposite material (b). The skin paw is adapted over the defect and sutured (c).

Histological processing of tissues.

Immediately after the death, the animals were decapitated and their heads were fixed en bloc in 10 % neutral formalin, rinsed in running water and demineralized in 8 % formic acid for five weeks. Through a series of transverse incisions, segments including critical calvarial defects and surrounding tissues including the nanocomposite material were selected, embedded in paraffin, cut into 6–8 μ m sections and stained with hematoxylin-eosin according to the standard technique. The examinations were carried out on Leica DM 5000B microscope.

Results

The empty defects in group 1 remained primarily empty throughout the study, demonstrating they were of critical size (non-healing throughout the studies). In contrast, the standardized calvarial

critical size defects filled with the composite paste based on NHACHCAPLLA at the 84 day were complete healed with newly developed connective and osteoid tissues which were ingrown between the implant components, forming a reticular pattern network (Fig. 2). There also was absence of acute and chronic inflammation or foreign body reaction. Other findings were visible biodegradation and mesh encapsulation of the implant components, as well as fibrous and osteoid tissue formation with well developed vascularisation and islets of newly formed bone, which all together fully filled the critical defects (Fig. 3 a, b).

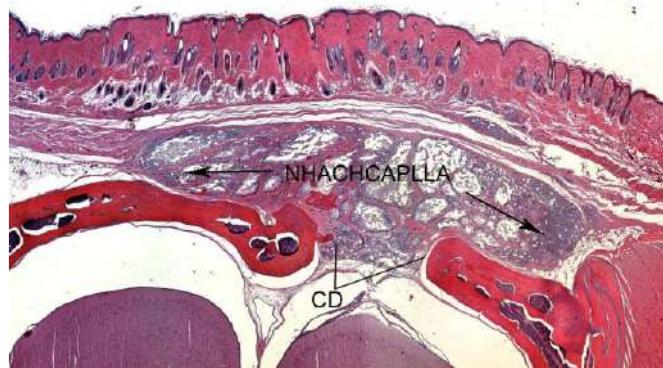


Figure 2: Standardized critical calvarial defect (CD) in a rat of at the 84th day following ther filling with composite paste containing nano-hydroxyapatite/ 3 % solution of chitosan in citric acid, and electrospinning fibres of poly lactic acid (NHACHCAPLLA). Complete healing with newly developed connective and osteoid tissues which in the form of a reticular pattern network are ingrown between the implant components. Hematoxylin-eosin.

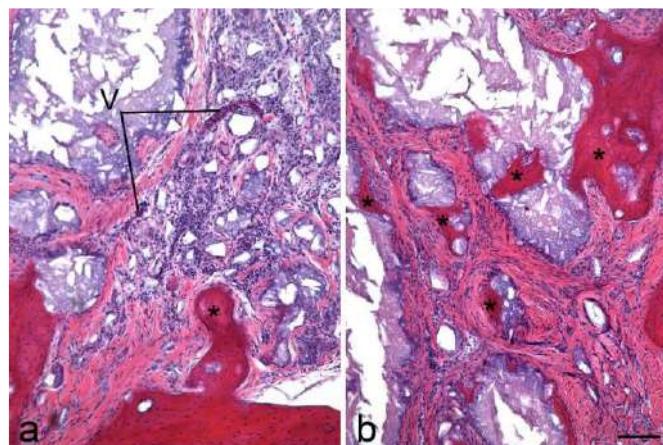


Figure 3: Fragments from fig.2 (a, b). Complete healing with newly developed connective and osteoid tissues which in the form of a reticular pattern network were ingrown between the implant components with well developed vascularisation (V in a) and islets of newly formed bone (asterisks in a, b). Hematoxylin-eosin. Bar = 100 μ m.

These results clearly show that the newly created composite paste is extremely useful for repairing of critical calvarial defects and most likely will find a wider application to other cases in bone augmentation surgeries.

Discussion

In the present study we analyzed the process of bone repairing in critical calvarial defects in rats treated with NHACHCAPLLA. For this purpose, we used critical size bone defects experimentally produced in rat calvaria as the biological model, which has been considered by many researchers to be the ideal model for testing bone graft materials in the craniomaxillofacial region [2, 4, 7, 8].

It should be emphasized that all animals presented an excellent response to implantation of the graft material, with the absence of inflammation during the study and a significant formation of healing tissues compared to control animals. Similar results were obtained and by other researchers used bone substitute grafts for stimulation of the healing of rat calvarial defects [3, 6, 9].

Conclusion

Based on the present results, we conclude that, with improvement in the quality control of the material production the NHACHCAPLLA will become a good alternative for the repair of bone defects in the calvarial region due to its capacity to allow the growing of newly developed connective and osteoid tissues which in the form of a reticular pattern network ingrown between the implant components with absence of acute and chronic inflammation or foreign body reaction.

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