

THE EFFECT OF CHITOSAN ON OSTEOGENESIS, HISTOLOGICAL STUDY IN RABBITS

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Abstract

Bone regeneration is a major limiting factor in oral surgery regenerative therapy. The concept of guided tissue regeneration has provided the strongest evidence that tissue healing can be influenced exogenously by wound management. At the cellular level, the existence of osteoprogenitors with the capacity to produce bone in the wound site and the potential to exogenously influence the behavior of these cells offers the opportunity to further enhance regenerative wound healing. Chitosan, with a chemical structure similar to hyaluronate, has been implicated as a wound healing agent. The purpose of this research was to evaluate the effect of chitosan on osteoblast differentiation and bone formation in vitro.

Introduction

This study aimed to test the bone healing capacity of chitosan by using it as a bone substitute material in rabbit jaw bone extraction sites.

The long term goal of this research is to investigate the optimal conditions for enhancing complete regeneration of periodontal bone defect extraction, auto-implant and alveolar ridge supporting tissues. The osseous tissue component is the main structural supporting tissue of the socket bone jaw and seems to determine the extent to which tissue regeneration will occur. For this reason, studies related to the activation and growth of bone forming cells are fundamental to the objectives of this research goal^{1,2}.

Bone regeneration is of particular interest, so this study investigates the effect of chitosan on osteoprogenitors differentiation and osteogenesis in vitro. The degree of alveolar ridge resorption becomes increasingly more problematic for patients wearing prosthetic tooth replacements, especially lower complete dentures. Dentures become less retentive

and more unstable as the severity of alveolar bone resorption continues.

Bone maintenance and bone regeneration have become essential concepts in the treatment of periodontal disease, in the healing of tooth extraction sockets and in the utilization of dental implants. Today, great efforts are being made not only to maintain and prevent bone loss but also to augment and regenerate bone around teeth and implants and to rebuild edentulous ridges³.

In order to understand why wound healing results in repair, or more importantly, how wound healing events can result in regeneration, one must consider the complexity of the cells and biomechanics involved extraction wound healing is a battle between cells with the potential to regenerate normal structures and forces that interfere with that process. The forces that interfere with regeneration include those that are exogenously introduced as well as those that are native to the wound healing environment⁴⁻⁸.

Cells with the potential to become cementoblasts and osteoblast are essential if true regeneration is going to occur. Once differentiated, these cells must be capable of movement, proliferation, production and intercellular communication. Cementoblasts, fibroblasts and osteoblast communicate with each other and are influenced by the extra cellular matrix and proteins which make up their environment. Any miscommunication between cells and influences from the extra cellular matrix may alter the wound healing pathway and lead towards repair rather than regeneration⁴⁻⁸. Like soft tissue wound healing, the healing process of bone fractures or defects is a complex and integrated sequence of events initiated by the stimulus of injury. Healing of a bone injury, like soft tissue, requires an integrated coordination between specialized cell types that work to restore structural and functional integrity. Specifically, three principle cells, osteoblasts, osteocytes and osteoclasts are involved in bone formation and remodeling⁹.

Osteogenesis and osteoconduction are the primary mechanisms by which bone grafts heal. Osteogenesis is the formation of new bone by surviving cells within the graft. Grafts must be well vascularized in order for bone forming cells within the graft to survive. Osteoconduction is the process by which blood vessels and cells grow into the graft and use it as a scaffold or matrix to form new bone. The process whereby dead bone is resorbed and replaced by new bone is referred to as "creeping substitution" by some investigators⁹.

Osteoinduction is the process of transformation of local undifferentiated cells into bone forming cells. The most notable example of an osteoinductive substance is the family of bone morphogenic protein or bone morphogenetic proteins. These proteins or

growth factors are released from the resorbing bone matrix and stimulate mesenchymal stem cells to differentiate and form bone⁹.

Biochemistry of Chitin and Chitosan

Chitin is second, only to cellulose, as the most abundant natural biopolymer. It is an important structural component of the exoskeleton of invertebrates (e.g. shrimp, crab, lobster), cell wall of fungi and the cuticle of insects. The very stable polysaccharide is a linear polymer of N-acetyl-D-glucosamine units joined in 1,4 β glucosidic linkages, the minimum descriptive unit being the disaccharide chitobiose. Chitin bears a close resemblance to cellulose, the major structural polysaccharide of plants which consists of D-glucose chains in 1,4 β linkages. Chitin, like cellulose, adopts a highly ordered chain conformation which gives rise to characteristic x-ray diffraction patterns¹⁰.

It was first prepared, at least in a concentrated form, by Braconnot in 1811 by the removal of the alkali-soluble material derived from some higher fungi¹⁰.

Chitosan is a derivative of chitin made by treating it with hot strong alkali. Analysis of this process has shown abundant deacetylation of side chains, which is manifest by a decrease in CO-OH bonds and an increase in OH-HO bonds. The resultant chitosan (1-4, 2-amino-2-deoxy- β -D-glucan) is a polycationic complex carbohydrate. Biodegradable and non-toxic, chitosan has a molecular weight of 800-1,500 Kd. Chitosan's availability in a variety of useful forms and its unique chemical and biological properties make it a very attractive biomaterial.

Chitin, which is processed to form chitosan, is most abundantly found in arthropod exoskeletons, fungi and plant cell walls. It is degraded via chitinase or lysozyme digestion. The degradation product is glucosamine which is a

natural monosaccharide that can be used by mammalian cells as an energy source. Chitosan's ability to be made into gels, films, membranes, fibers and beads as well as powders, flakes or solutions has led to many commercial and biomedical applications. This novel biomolecule may have many applications in dentistry and medicine due to its potential to effect the blood- tissue interface^{11,12}.

Material and Methods

This study was carried out as experimental study and conceded in College of Dentistry, Department of Oral Surgery, University of Basrah, from 1-8-2008 to 1-10-2008, 4 white healthy rabbits with an average age of 13 months. The weight of animals ranged between 2.5-3 kg and they were divided into; group A; two of these rabbits we create extraction of 2 lower anterior teeth and implanted with chitosan. group B; two of them also we do extraction but with out implantation with chitosan as control groups.

Preparation of chitosan

The raw material, exoskeleton of fresh prawn for preparation of chitosan was obtained from local market. The shells and head of fresh prawn were thoroughly and repeatedly washed in water and sun drained. The raw hydroxide and boiled for thirty minutes for deprotenization. After cooling the alkali is drained off and washed repeatedly and finally with ionized water to obtain neutral pH. The contents were transferred to a plastic container and five percent hydrochloric acid added and allowed to act for thirty minutes. The acid was decanted and repeatedly washed with water and then with ionized water. The excess water from the chitin obtained is removed by squeezing in a sterile lint cloth and air-dried. Chitin was immersed in forty percent sodium hydroxide and heated up to 90 degree Celsius for 90 minutes. The sodium hydroxide is quickly drained off

and the content washed repeatedly with water and finally with ionized water and the chitosan obtained was air dried after removing the excess water as above. The prepared chitin and chitosan was sterilized with ethylene oxide and packed in pre autoclaved polyethylene containers¹³.

Method

Following preparation of the operative field the rabbits receive;

Ketamine: the drug is given either intramuscularly in a dose of 30 mg/Kg or intravenous injection. Rabbits will respond to visceral pain but not to superficial pain under the influence of ketamine. The anesthetic time last between 30 and 45 minutes.

Xylazine (Kampon): the drug is given by intramuscular or intravenous routes. It induces a sedative hypnotic condition which is accompanied by a general muscle relaxation and in sensibility to pain (analgesia-anesthesia) IM route: 6mg/Kg, Iv route: 1.6 mg/Kg.

Atropine: are frequently given IM before anesthesia to decrease vagal cardiac intramuscularly (1ml).

Anesthesia: by giving an Im injection of ketamine 30 mg/Kg and Xylazine 6 ml/Kg atropine is used (one ml), and application of eye ointment to prevent dryness of the cornea. Post operative care and follow up, The animals were fed a green diet and received water and libitum, and oxytetracycline injected dose (2mg/day) of antibiotic intramuscularly after the operation.

After completion of extraction, the socket was irrigated by normal saline to confirm clean cavity. The chitosan powder was applied to the socket, wetted few drops of normal saline and condensed by plastic instruments with their mixture and then use sort by suturing the operation site by 0.5 silk and the wound washed with normal saline, then we give the rabbit atropine to compensate the action of ketamine.

The same procedure was done for control group. The lower anterior teeth and wound also closed by the same suture of operated group and we taken radiograph per apical type for both groups.

laboratory technique

The procedures were done by specialist histopathologist at private laboratory. the rabbits were scarified at 8 week, respectively after implantation. The mandibles were removed and fixed in 10% neutral buffered formalin (10 % N. B. F) for 5 days and refixed by mercuric chloride formalin for one day, the bone trim then decalcified in sodium citrate formic acid, the decalcification solution was changed every (48 h) till the bone becomes soft.

Tissue placed in decal solution must be washed in water for a minimum of 24 hours before processing.

Results

Control group

The slides are examined by two specialist histopathologist. In experimental socket cavities we found fibrovascular connective tissue. No

evidence of bone formation at this stage but an isolated foci of inflammatory reaction cells were detected with granulation tissue reaction (Fig. 1).

Periapical radiograph, post operatively follow up showing a crystal bone resorption with area of radiogralucency filled the defect area of extraction site in the same period and follow up of 2 groups.

Implanted group

Initial island bone proliferation were seen extending from the peripheral cavities of socket occupies the space under the chitosan particles with few collagen fibrous and new formation of blood vessels. osteoblast were observed but the osteoclast are not seen the chitosan particles covered with highly vascularized thin fibrous connective tissue (Fig. 2).

The radio graphical result (of operated group) periapical radiograph post operatively showing the level of crystal bone is still present with little or no bone resorption and the cavity is filled with scattered radio opacities (bone formation).

Tissue response	Control Group 4 week	Implanted Group 4 week
Loose fibrovascular tissue	Mild	Not found
New bone formation	Not found	Mild
Giant cell	Not found	Not found
Adipose tissue	Mild	Not found
Blood vessels	Not found	Mild
Connective tissue	Not found	Mild

Table I: Tissue response in the two groups

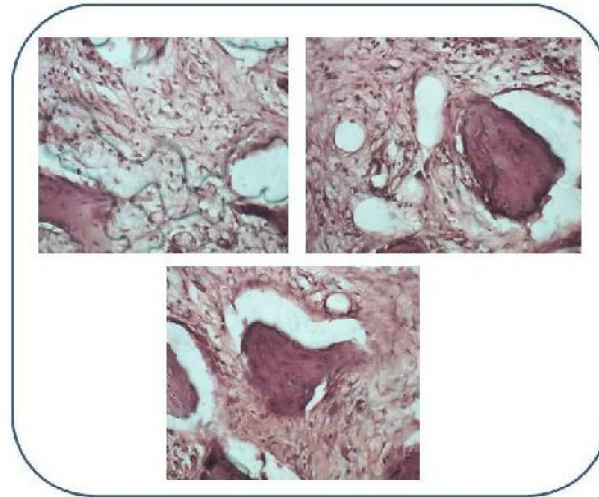


Fig. 1: Microphotograph of control group specimen after 4 weeks. showing the bundle of collagen fibers (connective tissue) toward the chitosan particle, with no newly bone formation .(Haematoxiline and eosin stain).

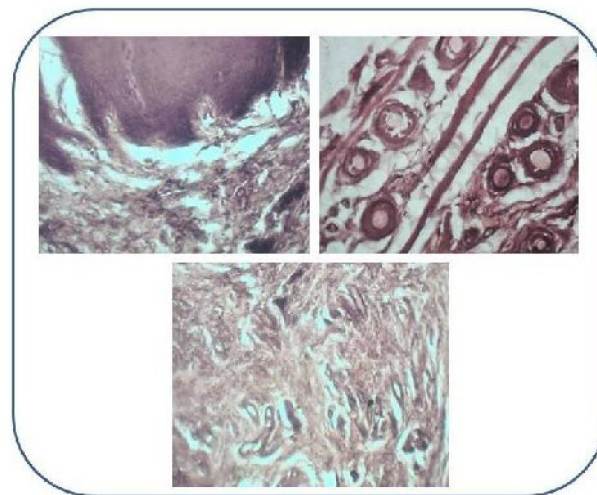


Fig. 2: Microphotograph of implanted group specimen after 4 week .showing the defect area between the periphery defect and the implant material filled by newly formed bone. (Haematoxiline eosin stain)

Discussion

Histological; In recent years, there was an increase in using the chitosan for hard tissue replacement and much hope rested on these materials. From this study, histological evaluation obtained that there is no evidence of inflammatory cells or rejection, of the tissue implanted with chitosan and we found no toxicity, nor foreign body response, which agree with the finding

of (Jardcho, 1980, Degroot, 1986, Kaneshigetel, 1996)^{14,15}.

The area under the grafted material gradually filled with newly bone formation adjacent to bone periphery of the defect cavities only with bundles of connective tissue fibers extending to surrounding implanted material and this tendency became more apparent with increasing time of implantation at 1,2,4 weeks respectively.

Clinically; In this study, the chitosan material was well tolerated in hard and soft tissue and does not seem to evoke any of the inflammatory responses and healthy during the course of follow up. Radiographic comparison of surgical site in grafted and control group progressive in the site of grafted material shaped as mention in radiographic result.

Conclusion

The chitosan material as substitute to bony defect characterized by, it enhance

healing by osteoconductive, and it safe material which aid in the regeneration of bone defect. The clinical study has demonstrated the chitosan is useful in regeneration and augmentation of bony jaw defect.

Suggestion

- 1- Further study in application of chitosan in large defect of jaw.
- 2- Further study for evaluation of chitosan for augmentation of resorbed ridge of jaw.

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