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Effect of small intestine sub mucosa in radius bone gap healing in rabbits

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Abstract

The present study was conducted to evaluate the efficacy of small intestine sub mucosa on enhancing healing of the experimentally induced bone gap in a rabbit model during the period from May 2009 to April/ 2010. A total of 16 healthy local rabbits with age of 7-9 months were used. The rabbits were divided into two equal groups: The individuals of the all groups were subjected to operation to produce bone gap (1cm) in the radius bone to get a critical bone defect. In the control group animals were left without any treatment. While in the treated group the bone defect was filled with small intestine sub mucosa which was harvested from sheep. Results revealed that there were no signs of immunological rejection or infection at the site of operation in all animals and the radiography showed a periosteal and endosteal reaction. Moreover the gaps were bridged faster in the treated group as compared with the control group. The histological examination showed a cartilage formation in a large amount on weeks (4 and 8) post operation in the treated group with wide bone trabeculae as compared to control group.

Keywords: Small intestine sub mucosa, Bone gap, healing

1. Introduction

The bone possess the capacity for regenerative growth and remodeling, both processes become impaired in clinical situations in which excessive bone loss is caused by disease, trauma or through tumor resection [1]. In order to address the need of the increasing number of patients who require bone for skeletal reconstruction, surgeons can overcome the disadvantages linked to auto or allograft by choosing the tissue engineering approach [2, 3].

Tissue engineering for bone faces the challenge of exporting successful laboratory protocols. The transfer of such technology for wide spread clinical using is still impractical.

Bio-derived materials and synthetic polymers made into three-dimensional porous structures possess some rigidity and have served as scaffolds in most traditional bone tissue. It is highly vascularized and relies on blood vessels to deliver nutrients and oxygen to cells deep in the mineralized bone matrix [4]. One of the greatest challenges faced in bone tissue engineering is how to enhance the supporting scaffold and newly formed bone tissue into establishing a natural capillary network [4]. The success of regenerating large volumes of bone which is valuable for clinical use depends on vascularization of these grafts. This still remains the greatest challenge for tissue engineering of bone.

The porcine small intestine sub mucosa (SIS) is a bio-derived multilaminar a cellular layer made up mostly of collagen that has shown to have characteristics that make it a proper material for tissue bioengineering in different anatomical sites [5]. There are several structural characteristics in SIS that are similar to periosteum, which make it optimal for using as a scaffold in the tissue – engineered bone membrane [6]. The membrane structure provides a large area for cells to attach and could permit cell survival through nutritional diffusion in the early stages before intrinsic angiogenesis, besides the membrane can guide the process of bone regeneration [7]. The objective of the present study was to evaluate the bone formation in the surgically created gap in diaphysis of radius bone in the rabbits by using sheep small intestine sub mucosa.

2. Materials and methods

The present study was conducted in Veterinary Medicine College in Baghdad, Iraq during the period from May 2009 to April/ 2010. Sixteen, (7-9) month old, healthy, local breed rabbits (1-1.5 kg in weight) were used in this study and kept in their cages. The rabbits were divided into two equal groups:

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Group I: 1cm bone gap was created at the mid shaft of radius bone and left without any treatment and considered as a control group.

Group II: a same bone gap was created and filled with sheep small intestine sub mucosa which prepared previously and considered as treated group.

The small intestine was harvested from the sheep and mechanically separating. The sub mucosa was taken from the outer muscular layers and the internal mucosal layer [8], then rinsed in highly purified water, treated with an aqueous solution of 0.1% per acetic acid for 2hr to ensure viral safety and then rinsed in sequential exchanges of water and buffers to yield a neutral ph. It was then freeze-dried to stabilize the proteins within it and sterilized using 70% ethanol alcohol for two hours [9]. Then it was rinsed with sterilized distilled water for (15 minute) then lyophilized the specimens (Fig.1).

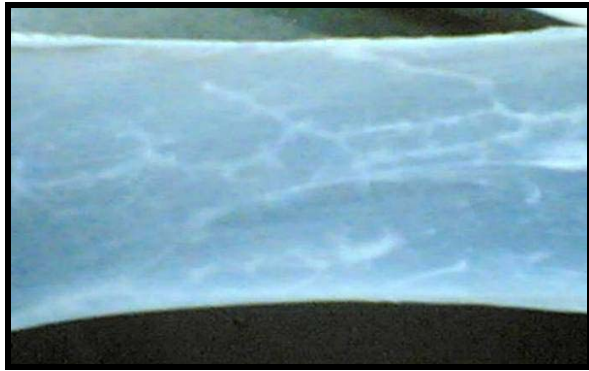


Fig 1: The small intestine sub mucosa after preparation

The animal was injected with Acepromazine melete (10 mg/kg BW.) I/M as a tranquillizer and after 10 minutes the animal was injected with a mixture of ketamine hydrochloride (35 mg/kg BW.) and xylazine (5mg/kg BW.) I/M [10]. The site of operation surgically prepared. The animal is casted in a lateral recompancy and made a surgical incision (3 cm) in length at the mid shaft of the radius from the medial aspect and opens the subcutaneous tissue. After that a blunt dissection between the pronator teres muscle and the flexor carpi radialis muscle- when the bone was exposed a 1cm bone gap - was done by using an electrical drill to create a critical bone gap. They washed with normal saline and filled with (SIS) in treated group. While in the control group the gap left without additives. Then the muscles were sutured with chromic catgut (3-0) to fixed the graft in its position. The skin was sutured with simple interrupted suture by using surgical silk (3-0) and the animal was given antibiotic for 5 days by using penicillin (10000 IU/kg BW.) and streptomycin (10 mg/kg BW.) I/M.

All animals subjected to radiography after (4, 8 weeks) post operation and were euthanized and the specimens were analyzed for the histological examination to notice the osteogenesis at the site of implantation.

3. Results

3.1 Clinical findings

All experimental animals appeared healthy, without any

complications (infection or rejection) in the site of operation, but the animals not bearing weight on the operated limb for about 3 days then the animals stand on their fore limb normally.

3.2 Radiological findings

At 4th week post operation there was no periosteal reaction with low opacity at the bone defect edge (Fig.2A), which continues until 8th week post- operation with slightly periosteal reaction at the defect edge which was clearly identified at this period (Fig.2B). In treated group at 4th week post-operation, more opacity was observed with periosteal reaction and deposition of osteoid bone at the edge of bone defect (Fig.2C), at 8th week post-operation the density of defect is similar to surroundings cortical part of bone with remodeling of marrow cavity and periosteal reaction at the defect site (Fig.2D).



Fig 2: Radiological appearance (A) Control group at 4th week post-operation, no periosteal reaction with low opacity at the edge of the defect. (B) Control group at 8th week post-operation, slight periosteal reaction at the edge of defect which clearly identified with low opacity. (C) Treated group at 4th week post-operation, more opacity observed at the site of bone defect with periosteal reaction at the edge of defect and deposition of osteoid bone at the texture of defect. (D) Treated group at 8th week post-operation, the density of defect is similar of surroundings cortical part of bone with remodeling of marrow cavity and periosteal reaction at the defect site.

3.3 Histological findings

The results of histopathology on 4th week post operation of the control group showed full the bone defect with organized clot, center of ossification with mature chondrocyte, deposition of granulation tissue and newly deposit of osteoid (Fig. 3A). The histopathology of the treatment group on 4th week post operation demonstrated. The trabecular bone completely bridged the bone defect with well-developed of haversian system and the remnant of small intestine sub mucosa scaffold, scanty of endochondral site of ossification for replacement tissue formed during the remodeling of bone matrix (Fig. 3B). Histopathological examination of the control group on 8th week post operation showed continues of ossification processes, newly trabecular bone formation and good osteoblastic appositional surfaces (Fig. 4A). While in the treated group after 8th week showed cortical bone formation, active deposit of osteoid surrounding of osteoblast and remodeling of cortical bone (Fig. 4B).

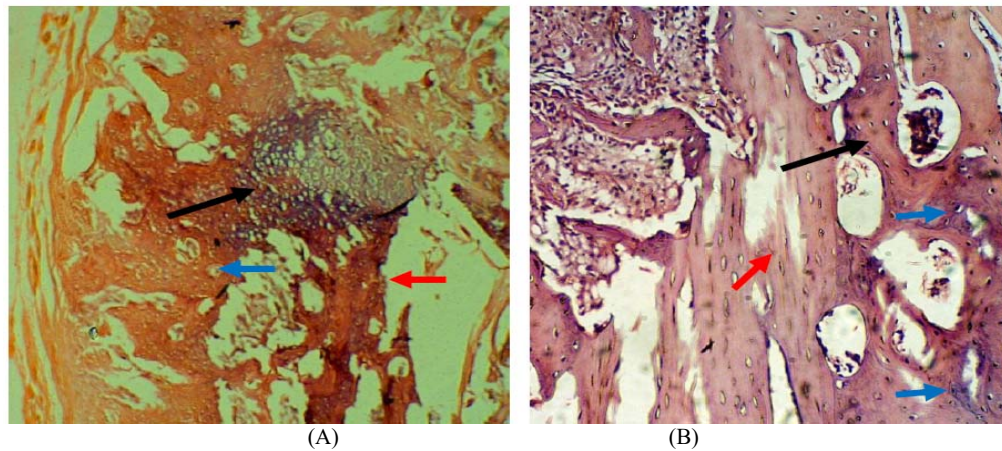


Fig 3: Micrograph showed radial bone defect after 4 weeks (A) Control group showed full the bone defect with organized clot (red arrow), center of ossification with mature chondrocyte (black arrow), deposition of granulation tissue and newly deposit of osteoid (blue arrow). (B) The treated group demonstrated trabecular bone (red arrow) completely bridged the bone defect with well- developed of Haversian system and remnant of small intestine sub mucosa scaffold (black arrow) scanty of endochondral site of ossification (blue arrow) for replacement tissue formed during the remodeling of bone matrix.

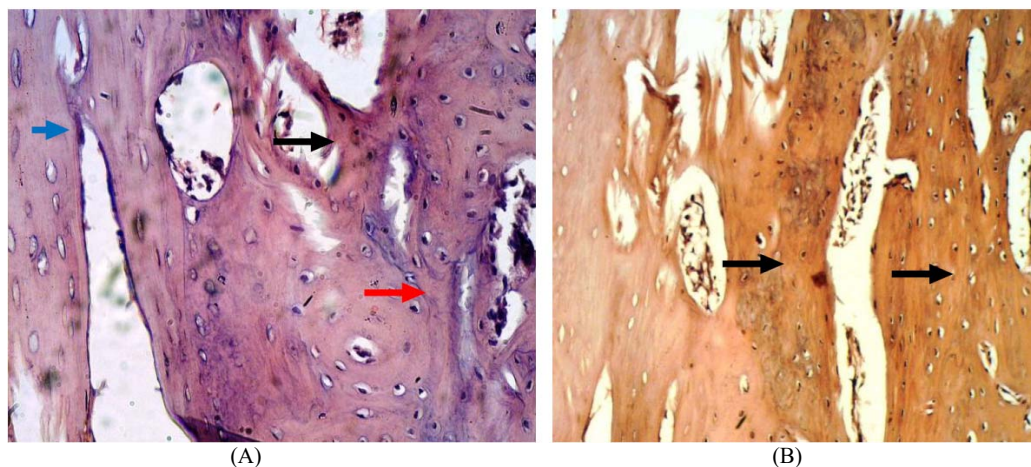


Fig 4: Micrograph showed radial bone defect after 8 weeks (A) in the control group showed continue of ossification processes (red arrow), newly trabecular bone formation (black arrow) and good osteoblastic appositional surfaces (blue head). (B) The treated group demonstrated cortical bone formation (arrow), active deposit of osteoid surrounding of osteoblast and remodeling of cortical bone.

4. Discussion

The present study showed that the small intestinal sub mucosa (SIS) is easily obtainable biomaterial, which composed of collagen (essential extra cellular matrix). It showed a resistant to infection and has a high degree of biocompatibility and strength. These results are similar to the results obtained by previous researchers^[11, 12] who used a scaffold successfully as a vascular graft material in the dogs. The clinical result of the present study showed no evidence of immunological rejection despite the derived from a xenogeneous animal and this seems to be in line with results of Lantz et al.,^[12] who concluded that this membrane induced an immune response in rats, activating T-helper-2cells. There was also a drop in the levels of inflammatory cytokines, as well as of the alpha tumoral necrosis factor so it didn't promote the rejections when implanted. The radiological findings of this study revealed a good bone formation in the treated group at 8 week post-operation as compared with control group in which the bone formation was slowly. These results are similar to results obtained by Murata et al.,^[13] who found that the small intestine sub mucosa induced bone conduction and thus facilitates the filling up of a bone defect that was surgically created. It acts as an optimal repair material and induced medullar cells growth in the graft bone neoformation, thus

promoting a fast cartilage formation. The histological findings revealed that the defect in the treated group was characterized initially at 4th week by large numbers of mononuclear cells and beginning cartilage formation. The specific reason for the presence of these cells is not known. Some authors suggested that these cells may play a role in filling of the bone defect with collagen^[11, 12]. In the mammals, transforming growth factor (TGF)-B is secreted by several cell types, including macrophages and lymphocytes^[14]. It has been capable of stimulating intra membranous bone formation and chondrogenesis^[15] and angiogenesis^[16] and of stimulating bone growth in animal models^[17]. Also the neovascular in cavities was within the newly formed bone tissue. This suggests that SIS degradation is relevant to angiogenesis and the mechanism is thought to be linked to the large amounts of vascular epithelial growth factor (VEGF) in the SIS^[18].

5. Conclusion

The present study showed that the small intestine sub mucosa facilitates increased the filling of bone gap defect in radius bone in rabbits. However, additional studies will be needed to evaluate the potential applications of SIS as a bone graft substitute.

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