GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

USING OF CHITOSAN AND AUTOLOGUS BONE MARROW IN RADIUS BONE GAP HEALING IN RABBITS

Omar Tariq Hammoodi
Department of surgery and obstetric College of veterinary medicine, University of Al-Anbar/Iraq

ABSTRACT
A study was conducted to investigate the effect of using chitosan and autologous in radius bone gap healing in rabbits. 10 local breed male rabbits in a good healthy were subjected to a surgical operation and divided randomly into two equal groups: Control group: a (1 cm) bone gap including the periosteum was induced in the radius bone to create a critical bone defect and leave it without any treatment. Treated group: a (1 cm) bone gap including the periosteum was induced in the radius bone to create a critical bone defect and filled with chitosan which prepared previously mixed with autologous bone marrow which harvested from the site of operation. All animals had a radiograph weekly and were killed after (2, 3, 6 weeks), the specimens were harvested and histological examination performed for evidence of osteogenesis. There are no signs of immunological rejection, no infection at the site of operation in all animals and the radiographic examination show high periosteal and endosteal reaction and the gap was bridged by bone callus formation in the treated group as compared with the control group. The histological examination shows a focal cartilage formation in large amount in week (3) and persists to the week (6) in treated group with wide bone trabecullae with observation of haversian and Volkmann's canal.

KEY WORDS: chitosan autologous rabbits.

INTRODUCTION

Bone fracture healing is a complex and dynamic regenerative process that gradually restores the structural integrity and mechanical function of bone. The healing process advances in stages of callus formation and consolidation and is eventually completed within some months[1]. A cancellous bone auto graft facilitates excellent bone formation, which can lead to bone union through its osteogenesis, osteoconduction and osteoinduction. It can be utilized to treat patients with nonunion, poor osteogenic potential, highly comminuted fractures and osteomyelitis. Hence, a cancellous bone autograftis still considered the ‘gold standard’ for bone graft. However, there are several disadvantages with this approach. These include a high risk of fracture and infection and pain at the donor site[2]. In addition, the amount of cancellous bone is limited. Therefore, many recent studies have focused on bone tissue engineering to over-come these disadvantages[3]. Bone tissue engineering involves the use of a combination of scaffolds with osteoblasts or osteogenic potential cells to form bone tissue, which can lead to new bone formation at the affected area when implanted in vivo. This attractive property of bone tissue engineering has resulted in consider-able developments in the field. However, the use of bone tissue engineering in clinical practice is still limited[4]. Chitosan is a polysaccharide biomaterial composed of glucosamine with variable levels of N-acetyl glucosamine that is biocompatible, cationic and adhesive, biodegradable, and angiogenic when implanted in bleeding wounds, Chitosan had been known to enhance tissue healing; it had been used in enhancement of bone healing[5] previously reported using animal cartilage repairmodels that chitosan-stabilized blood clot implants, when solidified in situ over micro fracture cartilage defects, elicit more trabecular bone and hyaline cartilage repair compared to controls[6-8]. Other studies have reported that insertion of an imidazole-modified chitosan sponge in osteochondral drill holes in sheep condyles led to a more complete bone repair after 20 and 40 days, compared to drilled controls [9], and that chitosan powder applied to canine bone fractures accelerated repair by approximately 1 week in veterinary practice[10]. Thus, current evidence indicates that chitosan in physical contact with bone marrow can stimulate osteogenesis in vivo, Osteogenesis during fracture repair occurs through endochondral ossification under hypoxic conditions, or through new woven bone deposition in vascularized granulation tissues[11].

The osteogenic capacity of bone marrow was first demonstrated in rabbits as early as 1869 by Goujon. Since2-4the 1960, some authors have shown that osteogenic stem cells in bone marrow are responsible for the biological efficacy of cancellous bone. This capacity has already been exploited, by several investigators, to reinforce the osteogenic properties of bone allograft by mixing the graft with bone marrow removed during surgery[12].

The aim of this study is to evaluate the effect of chitosan mixed with autologus bone marrow harvested from the site of operation to enhance bone gap healing in radius bone.

MATERIALS & METHODS

Preparation of chitosan by chemical methods

Chitosan was prepared by separation of crustacean shell and washed with water then dried by oven in (100°c) then crushed to powder, (100gm) was taken and mixed with (1L) of 5% (HCL) with shaking for 24hr in room

temperature for demineralization then purify and washed several times with distilled water to get rid of acid residue, then (1L) of 50% (NaOH) was added and heat the mixture at (90°c) for 3hr to deproteinization ,cool the mixture and washed the residue several times with distilled water to get Chitin, (20 gm)of chitin was taken and mixed with 250 ml of 50% (KOH) and heat with shaking at (90°c) for 6 hr. ,repeated this step three times then wash the exudes then dried the result was 75% of chitosan (13), Fig (1).

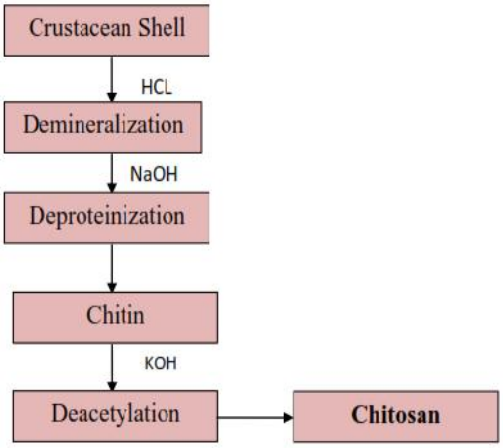

FIGURE 1: Preparation of chitosan

Surgical technique

Ten, (7-9) month old, healthy, local breed rabbits, 1.5-2 kg in weight were used in this study, the rabbits were divided into two equal groups:

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

Group Π: (1cm) bone gap was created at the mid shaft of radius bone and filled with chitosan powder which prepared previously mixed with bone marrow from the site of operation and considered as treated group.

The animal was injected with Acepromazine meleate (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), after the surgically prepared for the site of operation, the animal put in a lateral recombance and make a surgical incision (3 cm) in long, at the mid shaft of the radius from the medial aspect and open the subcutaneous tissue then a blunt dissection between the pronator teres muscle and the flexer carpi radialis muscle, when the bone was exposed a (1cm) long bone gap was created by using an electrical drill to create a critical bone gap, then washed with normal saline and filled with chitosan powder mixed with bone marrow from the site of operation in treated group, but in control group the gap left unfilled ,then the muscles was 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 3 days by using penicillin (10000 IU/kg BW.) and streptomycin (10 mg/kg BW.) I/M. All the animals had radiographs weekly and were killed after (2, 3, 6 weeks) and the specimens were harvested for the histological examination to notice the osteogenesis at the site of implantation.

RESULTS

Clinical findings

All animals remained healthy and did not have complications in their post-operative outcome at the site of operation, redness and the pain persisted for about 3 days then the animals stand on its fore limb normally.

Radiological findings

Control group

No evidence of bone gap filling was noted radiologicaly at the end of first week at the radius bone, at the end of second week we observed minimal periosteal reaction (Fig. 2), at the end of the third week the periosteal reaction continue with partial closure of the proximal end of bone gap which appear more radio-opaque and the complete closure of proximal end occur at the end of fourth week (Fig. 3), at the end of fifth week the partial bone filling continue at the proximal end but the distal end of bone gap persist open, at the end of sixth week the bone filling became more opaque at the bone gap with still partial open (Fig.4), at the end of eighth and ninth week apart of bone gap still open (Fig. 5), at the end of twelfth week the remain bone gap appear radio- opaque but the gap still distinguished (Fig. 6), these bone reactions continued until the eighteenth week we noticed complete closure of bone gap with obvious bone marrow cavity from one side only but at the end of nineteenth week we noticed complete healing of the bone gap with clearly obvious bone marrow cavity (Fig. 7).

Treated group

At the end of first week the induced gap at the radius bone was open and the chitosan powder with bone marrow appear transparent, the periosteal reaction noticed at the gap edges at the end of second week (Fig. 8), and these bone reactions increased at the end of forth week (Fig. 9), at the end of sixth and seventh week the endosteal reaction was observed and the bone formation appeared at the edges of bone gap at the end of seventh week (Fig.10), at the end of eighteenth week the bone callous formation increased and extend to the gap center which appear more radio-opaque (Fig. 11), the bone formation continued and filled all the bone gap at the end of nineteenth week with appearance of bone marrow cavity (Fig. 12).

FIGURE 2: minimal periosteal reaction second week in control group.

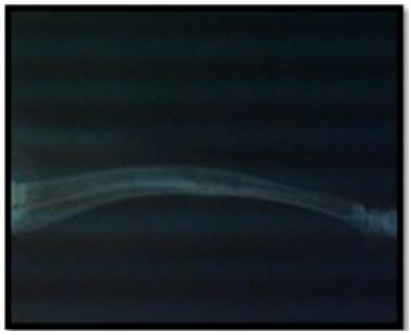

FIGURE 3: complete closure of proximal end occurs fourth week in control group.

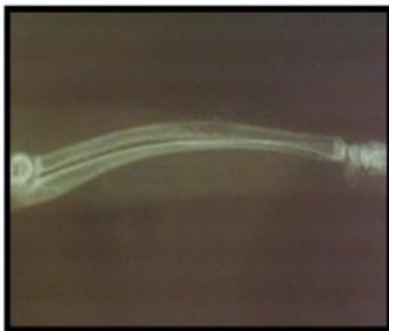

FIGURE 4: the bone filling became more opaque at the bone gap with still partial open sixth week in control group.

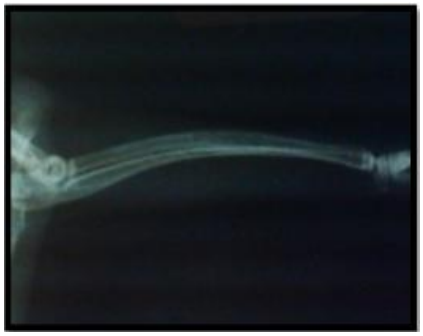

FIGURE 5: apart of bone gap still open ninth week in control group.

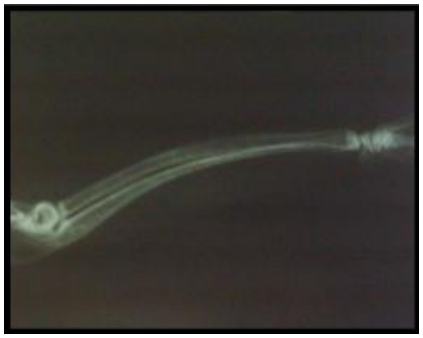

FIGURE 6: The remain bone gap appear radio- opaque but the gap still distinguished twelfth week in control group.

FIGURE 7: complete healing of the bone gap with clearly obvious bone marrow cavity at nineteenth week in control group.

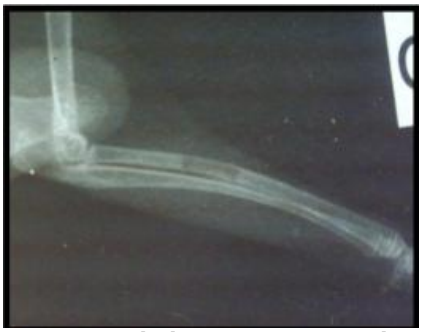

FIGURE 8: the bone gap was open, the periosteal reaction noticed at the gap edges second week in treated group

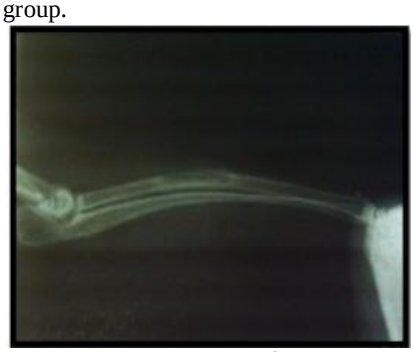

FIGURE 9: periosteal reactions increased at the edge of bone gap at forth week in treated group

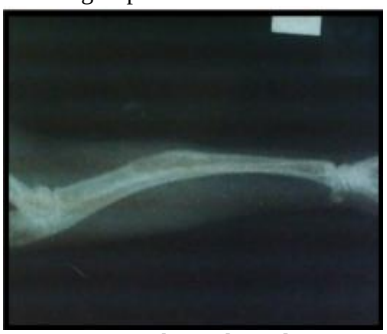

FIGURE 10: the endosteal reaction was observed and the bone formation appeared at the edges of bone gap at seventh week in treated group

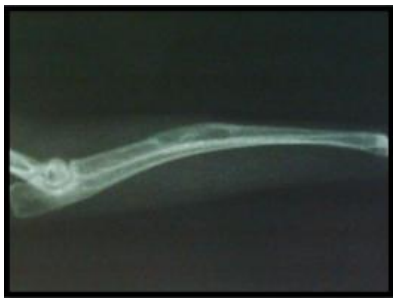

FIGURE 11: The bone callous formations increased and extend to the gap center which appear more radio-opaque at eighth week in treated group

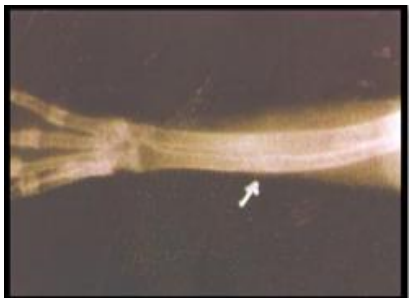

FIGURE 12: the bone formation filled the entire bone gap with appearance of bone marrow cavity at ninth week in treated group

Histological findings

Control group

At two week after operation we noticed irregular bone trabecullae which was spread indifferent directions, with the presence of osteoblast which located at the inner surface of bone trabecullae with the presence of multifocal cartilage formation (Fig.13), at three week the bone trabecullae extended with presence of hemorrhage (Fig.14), at sex week the bone trabecullae became larger and attached to each other (Fig.15).

Treated group

At two week we noticed highly infiltration of mononuclear inflammatory cells around the implant material with the presence of bone trabecullae and we observed the osteoblast and the osteoclast cells (Fig.16), at three week the bone trabecullae became wider and more extended with presence of large focal of cartilage formation with highly blood vessels (Fig.17), at week sex the bone trabecullae became regular and thick and directed towered the intact bone (Fig.18).

DISCUSSION

Research on biomaterials for bone implantation and replacement has expanded considerably over the last four decades. In recent years, significant progress has been made in organ transplantation surgical reconstruction and the use of artificial prostheses to treat the loss or failure of an organ or bone tissue. The establishment of a load bearing biomaterial must be incorporated with natural bone. The implanted biomaterial should possess the following criteria biocompatibility, osteoconductivity, high porosity and biomechanical compatibility[14]. Chitosan has played a major role in bone tissue engineering over the last two decades, being a natural polymer obtained from chitin, which forms a major component of crustacean exoskeleton. In recent years, considerable attention has been given to chitosan composite materials and their applications in the field of bone tissue engineering due to its minimal foreign body reactions, an intrinsic antibacterial nature, biocompatibility, biodegradability, and the ability to be molded into various geometries and forms such as porous structures, suitable for cell in growth and osteoconduction [15]. The radiological findings of this study revealed a good bone formation in treated group at nine week as compared with control group in which the bone formation was slowly and progressive and completed at 19 week, according to Venkatesan, J. and Kim, S. (2010), found chitosan with composites to be potential bone implant material with good osteoconductive, osteiinductive and osteogenic properties which facilitates the filling up of a bone defect that was been surgically created. The histological findings revealed that the treated group defect was characterized initially at 3 weeks by large numbers of mononuclear cells and beginning cartilage formation, the specific reason for presence of these cells is chitosan particles themselves did not mineralize; they accumulated intra- and extracellularly where they were space-filling and excluded the deposition of mineralized collagen fibrils. Chitosan particles did result in many chitosan-cell complexes that may have sterically interfered with interactions between osteoblasts, collagen fibrils, and accessory proteins needed for mineral nucleation, maturation or stability. Chitosan stimulate osteoblast differentiation through a non-specific increase in cell adhesion. In this study, chitosan added in particulate form did not increase attachment of cells to the tissue-culture plastic. Chitosan particles also failed to stimulate the release of angiogenic factors. Altogether, these data allow some authors to reject the hypothesis that chitosan particles promote osteogenesis by directly stimulating BMSCs to differentiate or to release angiogenic factor [16].

Bone marrow is known to be osteogenic immediately after bone grafting it contains osteogenic cells which when grafted orthotopically, respond to the osteoinductive stimuli present by producing bone.

In summary, the combination of chitosan powder with bone marrow facilitates rapid filling of bone gap defect in radius bone in rabbits, suggesting that filling is the result of osteoconductive and osteoinductive processes.

REFERENCES

[1]. Einhorn, T. A. (1995) Enhancement of fracture-healing. J. Bone joint Surg. Am., 77(6), pp: 940–956.

[2]. Martinez S.A., Walker T. (1999) Bone grafts. Veterinary Clinics North America Small Animal Practice, 29, 1207–1219.

[3]. Drosse I., Volkmer E., Capanna R., De Biase P., Mutschler W., Schieker M. (2008) Tissue engineering for bone defect healing: an update on a multi-component approach. Injury, 39 Suppl. 2, S9–S20.

[4]. Cancedda R., Giannoni P., Mastrogiacomo M. (2007) A tissue engineering approach to bone repair in large animal models and in clinical practice. Biomaterials, 28, 4240–4250.

[5]. AL-Robaee, A. (2005) Radiological and histopathological studies of the effect of Chitosan implant on healing of fractures induce in the distal part of the tibia in rabbit model. Master thesis of veterinary medicine university of Basrah.

[6]. Hoemann, C.D., Sun, J., McKee, M.D., Chevrier, A., Rossomacha, E., Rivard, G.E., Hurtig, M., Buschmann, M. D. (2007) Chitosan- glycerol phosphate/ blood implants elicit hyaline cartilage repair integrated with porous subchondral bone in microdrilled rabbit defects. *Osteoarthr Cartil* 2007, 15**:**78-89.

[7]. Hoemann, C.D., Hurtig, M., Rossomacha, E., Sun, J., Chevrier, A., Shive, M.S., Buschmann, M.D. (2005) chitosan- glycerol phosphate /blood implants improve hyaline cartilage repair in ovine microfracture defects.J.bone joint surg.am;87:2671-86.

[8]. Chevrier, A., Hoemann, C.D., Sun, J., Buschmann, M.D. (2007) Chitosan-glycerol phosphate/blood

implants increase cell recruitment, transient vascularization and sub-chondral bone remodeling in drilled cartilage defects. Osteoarthr Cartil; 15:316–27.

[9]. Muzzarelli, R.A., Mattioli-Belmonte, M., Tietz, C., Biagini, R., Ferioli, G., Brunelli, M. A., Fini M., Giardino, R., Ilari, P., Biagini, G. (1994) Stimulatory effect on bone-formation exerted by a modified chitosan. Biomaterials. 15:1075–81.

[10]. Khanal, D.R., Choontanom, P., Okamoto, Y., Minami, S., Rakshit, S.K., Chandrakrachang, S. Stevens, W.F. (2000) Management of fracture with chitosan in dogs. Indian Vet. J. 77:1085–9.

[11]. Carano, R.A., Filvaroff, E.H. (2003) Angiogenesis and bone repair. Drug Discov Today; 8:980–9.

[12]. Boehm, C.A., Muschler, G.F. (1999) Rapid concentration of bone marrow derived osteo-blastic progenitor cells (CFU-Os) in allograft bone powder . J Bone Miner Res.; 14 (Suppl D): S474.

[13]. Granja, P. L. and Silva, A. I. (2003) Preparation and characterization of injectable chitosan hydroxyapatate microspheres. Htt://www.scientific net./A:/composite. Htm (internet).

[14]. Pizzoferrato, A., Cenni, E., Ciapetti, G., Granchi, D., Savarino, L., Stea, S. (2002) Inflammatory response to metals and ceramics. In Integrated Biomaterials Science; Kluwer Academic/Plenum Publishers: New York, NY, USA; pp. 735–791.

[15]. Venkatesan, J. and Kim, S. (2010) Chitosan composites for bone tissue engineering- An overview. Marine Drugs. 8, 2252-2266.

[16]. Jessica Guzmán-Morales, Hani El-Gabalawy, Minh H. Pham, Nicolas Tran-Khanh, Marc D. McKee, William Wu d, Michael Centola, Caroline D. Hoemann (2009) Effect of chitosan particales and dexamethason on human bone marrow stromal cell osteogenesis and angiogenic factor secretion. Bone, 45:617-626.