

Ultrasonographical and Radiological Evaluation of the Cancellous Bone Scaffold Coated with Nano-Hydroxyapatite in Repair of Radial Bone Defect in Rabbit

¹Rasoul Rahimzadeh, ²Abbas Veshkini, ³Davood Sharifi, ³Gholamreza Abedi

¹Department of Radiology, Faculty of Veterinary Science, Science and Research Branch, Islamic Azad University, Tehran, Iran.

²Department of Radiology, Faculty of Veterinary Science, Science and Research Branch, Islamic Azad University, Tehran, Iran.

³Department of Surgery, Faculty of Veterinary Science, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Abstract: To evaluate the osteo-regenerative capacity of proprietary bone grafting material as a bone defect filler and osteogenetic stimulation to speed up bone healing too. In this study eighteen adult male New Zealand white rabbits were anesthetized and a segmental full thickness bone defect of 10 mm in length was created in the middle of the right radial shaft in all rabbits. They were divided into two groups of 9 rabbits. Group I was considered as control and the fractured site was fixed using finger bone plate with 4 screws, whereas the cancellous bone scaffold coated with Nano-Hydroxyapatite was used to fill the gap after fracture fixation in Group II. Radiography, two dimensional and color doppler ultrasonography were done before and after creating defects and on 0, 15, 30, 60 and 90 days to evaluate local reaction as far as new blood vessels network and callus formation are observed. On the radiographs during the whole process, bone repair in Group I was not as perfect as those in Group II samples and trace of internal callus filled the gap incompletely in 60 days in Group I, whereas in Group II internal callus almost was formed on 30 days and in addition intercortical callus was seen supporting to cover and filled the gap completely in this group in 60 day; Sonographic findings confirmed the protrusion of newly formed blood vascular network in 30 days in Group I and from 15 days in Group II and remarkably increased till end of observation period. These results indicate that the Nano-Hydroxyapatite with more features and shorter in time, made possible the reconstruction of bone tissue and alternative techniques as well as previous bone graft, also radiography and ultrasonography are reliable techniques to trace local reaction at proper time.

Key words: Nano-hydroxyapatite , Ultrasonography, Radial bone, Radiography, Vascular network.

INTRODUCTION

One of the clinical problems in humans and animals are healing fractures, considering the non healing hypothesis and long term healings many investigations have been performed about the case in recent decades. The main attempts of these investigations were precipitation in healings, osteopathy and battening capability (Morgan and Leighton, 1995). All kinds of osteografts assimilate in body and assimilation processes in spongy bones are faster than compact bones (Davies, 1998; Remedios, 1999; Salgado *et al.*, 2004). Bone can usually heal itself, but in traumatic cases, like cancer or a crushed limb, there's often an extensive amount of damage. The cancellous bone scaffold would help in these situations by bridging the missing area until new bone could grow to replace it (Sommerfeldt and Rubin, 2001). In the application of bone defect repairing material, it is desired that the structure be simulated, imitated, or replicate the porous structure of biological bone, so that it can adapt to the stress changes in a certain range, circulate blood well, ensure normal growth and metabolism of bone tissue, and accelerate bone reconstruction (Zhu *et al.*, 2007). Nanohydroxyapatite (nano-HA), materials show similar chemistry to the bone apatite and depending on the underlying topography and the method of preparation, the nanohydroxyapatite may simulate the specific arrangement of the crystals in bone. Hydroxyapatite (HA) and other calcium phosphate materials have been indicated in cases in which the optimal surgical fit is not achievable during surgery, and the HA surface properties may enhance bone filling of the defect area (Meirelles, 2008). The biological properties of the synthetic hydroxyapatite are well

Corresponding Author: Rasoul Rahimzadeh, Department of Radiology, Faculty of Veterinary Science, Science and Research Branch, Islamic Azad University, Tehran, Iran.
Email: Drtwor@gmail.com<mailto:Drtwor@gmail.com>

documented in literature, and it has been used for correcting bone defects in human beings and in other species (Geesink *et al.*, 1995; Kveton *et al.*, 1995; Ben-Nissan and Choi, 2006). with results good enough for indicating it as an alternative for bone graft in clinical practice. The normal healing process of fractured bones can be visualized using power Doppler ultrasonography and radiography and follows a distinctive time-dependent pattern. This investigation evaluates the cancellous bone scaffold coated with nano-HA using ultrasonographic and radiographic techniques.

MATERIALS AND METHODS

This study was performed in accordance with the Islamic Azad University Law on animal experimentation. Rabbits were treated accordingly to animal welfare legal regulations. Eighteen adult male New Zealand rabbits having body weight from ranged from 3.0 - 3.5 Kg were given general anesthesia using combinations of acepromazine 1 mg kgbw⁻¹ intramuscularly as premedication (Karon Co. Iran) and anesthesia was induced intramuscularly using ketamine 35 mg Kg⁻¹ (Alfasan Woerden- Holland) with combination of xylazine 5 mg Kg⁻¹(Afasan Woerden-Holland). Under restrict aseptic condition, skin incision was given on the latero medial surface of right radial bone and after separation of connective tissues and fascia a segmental full thickness bone defect of 10 mm in length was created in the middle of the right radial shaft in all rabbits (Fig. 1). They were divided into two groups of 9 rabbits each. Group (I) was considered as control and the fractured site was fixed using finger bone plate with 4 screws, whereas the cancellous bone scaffold coated with Nano-Hydroxyapatite was used to fill the gap after fracture fixation in Group (II) (Fig. 2). Radiography and two dimensional and color Doppler ultrasonography were done before experiment, after fixation and on 15, 30, 60 and 90 days and interpretation were done by considering dislocation of graft, new-bone formation and new blood vascular network formation.

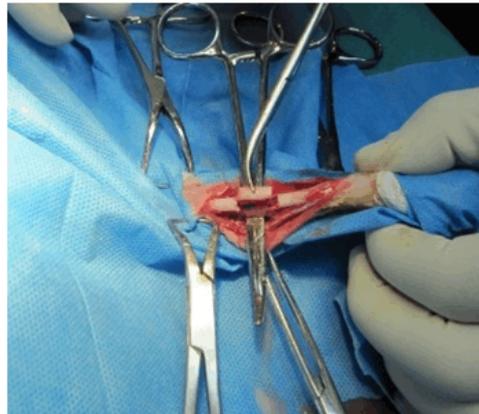


Fig. 1: Removal of cut piece of bone.



Fig. 2: Fixation of radial bone and packing of defect using Cancellous Bone Scaffold Coated with nano-HA.

Post-operative Care:

Antibiotics, penicillin G procaine 40000 IU kg⁻¹, I.M. bid dexamethasone 0.6 mg Kg⁻¹, Vitamin B Complex 0.2 mg Kg⁻¹ and analgesic agent such as Tramadol hydrochloride 5 mg Kg⁻¹, i.m. bid were administered for 3 post-operative days. The lateral and cranio-caudal radiographs were taken before experiment, after fixation and on 15, 30,60 and 90 days:

- Radiographic evaluation was done using the healing indices such as:
- The probable inchoate internal callus
- The probable inchoate external callus
- The probable inchoate intercortical callus
- Osteo inductions in adjacent bones
- The amount of scaffold opacity in defective bone and its role in osteoconductivity during healings process
- The amount of healing callus in defective bones
- Sonographic evaluations were performed by Sonosite Titan Equipment. Linear probe in 10 Megahertz frequency and imbuing topical gel used in ultrasonography.

Results:

No operative or postoperative complications were encountered. All of the rabbits tolerated operation well and survived until the final study period. No wound opening or infections were observed. Radiographically keeping in view the parameters were used to evaluate the major changes revealed no sign of infection on the edges of each defect and both groups permitted stabilization of the defected bones and maintenance of correct position. The new bone formation and local reaction in form of biodegradation of cancellous bone scaffold coated with nano-HA in the bone defects in Group II could be clearly reflected by the gradually replacing with new bone invasion. On the radiographs during the whole process, bone repair in Group I (Fig. 3) was not as perfect as those in Group II samples (Fig. 4) and trace of internal callus filled the gap incompletely in 90 days in Group I, whereas in Group II internal callus almost was formed on 30 days and in addition intercortical callus was seen supporting to cover and filled the gap completely in this group (Fig. 4, Table 2) Radiographs obtained in the experimental group showed that the radiopacity of scaffold used in gap was non-uniform but so that non-uniformity in the three months after surgery decreased gradually. Ultrasonographic findings confirmed the protrusion of newly formed blood vascular network in 30 days in Group I (Fig. 5, Table 3) and from 15 days in Group II and remarkably increased till end of observation period (Fig. 6, Table 4). No Doppler signal was present during the first 15 days. The mean of the scores was highest between 20 and 30 days postoperatively and the median of the scores peaked between 31 and 40 days.

Table 1: Radiographic findings in Group I at different intervals.

Day	0	15	30	60	90
Soft Tissue	Swelling Soft Tissue	Decrease of Swelling Soft Tissue	No Swelling Soft Tissue	No Swelling Soft Tissue	No Swelling Soft Tissue
Osseous Tissue	1.No Internal callus 2.No External callus 3. No cortical callus 4. To empty defect from callus 5.No osteo Induction	1.No Internal callus 2.No External callus 3. No cortical callus 4. To empty defect from callus 5.No osteo Induction	1.No Internal callus 2.No External callus 3.No InterCortical callus 4. To empty defect from callus 5.No osteo Induction	1. Internal callus 2.No External callus 3.No InterCortical callus 4. Relative to fill defect from callus 5.No osteo Induction	1. Internal callus 2.No External callus 3.No InterCortical callus 4. Relative to fill defect from callus 5.No osteo Induction

Table 2: Radiographic findings in Group II at different intervals

Day	0	15	30	60	90
Soft Tissue	Swelling Soft Tissue	Decrease of Swelling Soft Tissue	No Swelling Soft Tissue	No Swelling Soft Tissue	No Swelling Soft Tissue
Osseous Tissue	1.No Internal callus 2.No External Callus 3. No InterCortical callus 4. To empty defect from callus 5.No osteo Induction	1.No Internal callus 2.No External callus 3.No InterCortical callus 4. To empty defect from callus 5.No osteo Induction	1. Internal callus 2.No External callus 3.No InterCortical callus 4. To empty defect from callus 5.No osteo Induction	1. Internal callus 2.No External callus 3. the cortex was bridged with newly formed bony tissue 4. Completly to fill defect from callus 5.No osteo Induction	1. Internal callus 2.No External callus 3. InterCortical callus 4.Completly to fill defect from callus ,Callus uniformity was increased 5.No osteo Induction

Table 3: Findings of two dimensional sonography and color Doppler in Group 1

Day	0	15	30	60	90
Ultrasonography B-mode	Defect : Hypo Echogenicity	Defect : Hypo Echogenicity	Defect : Increase Echogenicity	Defect : Increase Echogenicity	Defect : Increase Echogenicity
Oseous Tissue					
Ultrasonography	Defect : No	Defect :No	Defect: small	Defect: Many	Defect : Decrease
Color Doppler	Vascular Blood	Vascular Blood	Vascular Blood	Vascular Blood	Vascular Blood

Table 4: Findings of two dimensional sonography and color Doppler in Group 2.

Day	0	15	30	60	90
Ultrasonography B-mode	Defect : Hypo Echogenicity	Defect : Increase Echogenicity	Defect : Increase Echogenicity	Defect: Hyper Echogenicity	Defect: Hyper Echogenicity
Oseous Tissue					
Ultrasonography	Defect : No	Defect : Small	Defect: Many	Defect: Decrease	Defect : Small
Color Doppler	Vascular Blood	Vascular Blood	Vascular Blood	Vascular Blood	Vascular Blood



Fig. 3: Radiograph of group I at 90 days.



Fig. 4: Radiograph of group II at 90.

Discussion:

Radiology is a most important diagnostic test in the investigation of skeletal disease because good radiographic contrast is naturally provided between the bone and surrounding soft tissues permitted detection of rather small but clinically important changes (Barden and Brinker, 1976). Ultrasonography and the Doppler effect are noninvasive and inexpensive method of investigation, they are full of prospect for the monitoring of bone healing after fracture (Elanga *et al.*, 1997). The nano-HA is similar to the mineral part of natural bone in both composition and structure (Du *et al.*, 2000; Liao and Cui, 2004). It can simulate the metabolism of nature bone and is good for new bone formation. The features of the nano-HA provid ideal frame to guide the new bone formation, so it promotes the reparative process of bone defect (Meirelles, 2008). As per expected no local reaction was seen during first two weeks in both groups but sonographically sprouting small

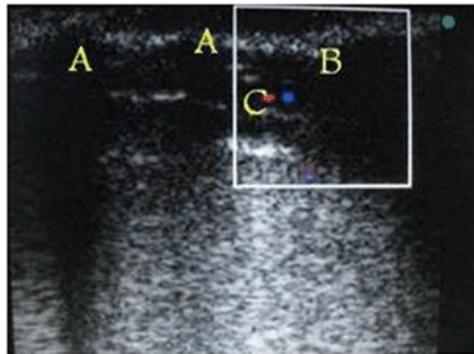


Fig. 5: Sonogram of group I showing (A) screw; (B) defect; (C) Vacular Blood.

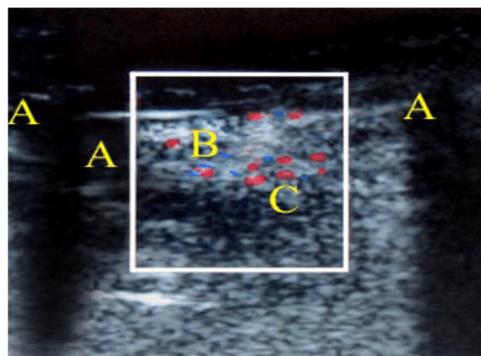


Fig. 6: Sonogram of group II showing (A) screw; (B) defect; (C) Vacular Blood.

blood vessels network at end of two weeks in Group II with radiographic findings of intercortical callus formation within 60 days were good indication for local reaction of cancellous bone scaffold coated with nano-HA to act as stimulator to provide suitable bed for blood vessels penetration as compared to Group I similar to observations of (Williamson *et al.*, 1991; Hamanishi *et al.*, 1994; Protopappas *et al.*, 2008; Tikku *et al.*, 2010). The status of bone, the vascularity, callus formation and viability of bone is a very important factor, in assessing the successful outcome of various surgical procedures. This information is not offered by routine radiographs, which cannot detect bony changes before 4 weeks. Ultrasonography is a reliable alternative, to study the callus formation. Early stages are seen as hypoechoic areas with flecks of calcification seen as hyperechoic structures These results also agree with those concluded by (Craig *et al.*, 1999) Critical evaluation of radiographs taken at different intervals in the animals of group I revealed moderate evidence of fracture union as compared to the other group. However, in the initial stages, minimal Internal callus and smoothing edges of the cortical bone defects were noticed. This may be due to the larger defect size, which is in agreement with the observations of Singh (1998). In the animals of group II, day 0 radiographs revealed the presence of well-placed cancellous bone scaffold in the mid-shaft radial diaphyseal defects which were relatively distinguishable to the radio-density of host bone Between days 15 to 30, there were well-established Internal callus without any signs of scaffold resorption. On day 30, the cancellous bone scaffold were in the process of resorption from all four corners and the it were replaced by radiologically-detectable newly grown bone, which is in agreement with other observations (Singh, 1998; Nandi *et al.*, 2008). On day 60, the cortex of the defect along the longitudinal axis was bridged with newly formed bony tissue, indicating a well-organized healing process. Complete bridging of the cortical defect was observed on day 90 with similar radio-dense bony material and the scaffold was encapsulated. Evaluation of ultrasonographic results of the present study revealed varying degrees of vascularization. However, the evidence of vascularization was more pronounced in animals implanted with cancellous bone scaffold coated with nano-HA than the controls. The minute vessels of periosteal and endosteal origin invading the implant bed supports the view that vascularization in fracture healing is directly related to the amount of new bone formation correlate with the findings reported by (Protopappas *et al.*, 2008; Zhu *et al.*, 2009). Findings of this study confirmed biological

activity of scaffold despite of having self-blood vessels limitation. considering that bone is a natural nanostructure so it seems Cancellous bone scaffold coated with nano-HA fill the deficit of local reaction to be effective.

Conclusion:

Because the Cancellous bone scaffold coated with nano-HA mimics bone so closely, it not only replaces the missing section of bone, but promoted bone formation over the defect, conforming their biological osteoconductive property and radiography and ultrasonography are reliable techniques to trace local reaction at proper time.

Abbreviations: nano-HA, Nano-hydroxyapatite; HA, hydroxyapatite.

REFERENCES

- Barden, T.D. and W.O. Brinker, 1976. Radiologic and gross anatomic evaluation of bone healing in dog. *J. Am. Vet. Med. Assoc.*, 169: 1318.
- Ben-Nissan, B. and A.H. Choi, 2006. Sol-gel production of bioactive nanocoatings for medical applications. Part 1: an introduction. *Nanomedicine*, 1(3): 311-319.
- Craig, J.G., J.A. Jacobson, B.R. Moed, 1999. Ultrasound of fracture and bone healing. *Radiologic. Clinics of North America*, 37: 737-751.
- Davies, J.E., 1998. Mechanisms of Endosseous Integration. *Int. J. Prosthodont*, 11(5): 391-401.
- Elanga, M., B. Bouche, P. Putz, N. Dumont, 1997. Ultrasonography and Doppler effect, an original method for the early and dynamic evaluation of bone callus. *Acta. Orthop. Belg.*, 63(4): 233-9.
- Geesink, R.G.T., N.H.M. Hoefnagels, 1995. Six-year results of hydroxyapatite-coated total hip replacement. *J. Bone. Joint. Surg. Br.*, 77: 534-47.
- Hamanishi, C., T. Yosii, S. Tanaka, 1994. Maturation of the distracted callus. Sonographic observations in rabbits applied to patients. *Acta. Orthop. Scand*, 65: 335-338.
- Kveton, J.F., C.D. Friedman, P.D. Costantino, 1995. Indications for hydroxyapatite cement reconstruction in lateral skull base surgery. *Am. J. Ontol.*, 16: 465-9.
- Liao, S.S., F.Z. Cui, 2004. In vitro and in vivo degradation of mineralized collagen-based composite scaffold: nanohydroxyapatite/collagen/poly(L-lactide). *Tissue Engineering*, 10(1-2): 73-80.
- Meirelles, L., T. Albrektsson, P. Kjellin, A. Arvidsson, V. Franke-Stenport, M. Andersson, F. Currie, A. Wennerberg, 2008. Bone reaction to nano hydroxyapatite modified titanium implants placed in a gap-healing model. *J. Biomed. Mater. Res.*, 87(A): 624-31.
- Meirelles, L., A. Arvidsson, M. Andersson, P. Kjellin, T. Albrektsson, A. Wennerberg, 2008. Nano hydroxyapatite structures influence early bone formation. *J. Biomed. Mater. Res.*, 87(A): 299-307.
- Morgan, J.P., R.L. Leighton, 1995. Radiographic appearance of fracture healing. in: *Radiology of small animal fracture management*. Philadelphia. WB Saunders Co, 34-43.
- Nandi, S.K., B. Kundu, S.K. Ghosh, D. Kumar De, D. Basu, 2008. Efficacy of nano-hydroxyapatite prepared by an aqueous solution combustion technique in healing bone defects of goat. *J. Vet. Sci.*, 9(2): 183-191.
- Protopappas, V.C., M.G. Vavva, D.I. Fotiadis, K.N. Malizos, 2008. Ultrasonic Monitoring of Bone Fracture Healing. *IEEE. Trans. Ultrason. Ferroelectrics. Freq. Contr.*, 55(6): 1243-1255.
- Remedios, A., 1999. Bone and bone healing. *Vet. Clin. North. Am. Small. Anim. Pract.*, 29: 1029-44.
- Salgado, A.J., O.P. Coutinho, R.L. Reis, 2004. *Bone Tissue Engineering: State of the Art and Future Trends*. *Macromol. Biosci.*, 4: 743-765.
- Singh, S., 1998. Reconstruction of segmental ulnar defect with tricalcium phosphate, calcium hydroxyapatite and calcium hydroxyapatite- bone matrix combination in rabbit, MV Sc Thesis, Indian Veterinary Research Institute., Izatnagar. India.
- Sommerfeldt, D.W., C.T. Rubin, 2001. Biology of bone and how it orchestrates the form and function of the skeleton. *Eur. Spine J.*, 10: S86.
- Tikku, A.P., S. Kumar, K. Loomba, A. Chandra, P. Verma, R. Aggarwal, 2010. Use of ultrasound, color Doppler imaging and radiography to monitor periapical healing after endodontic surgery. *Journal of Oral Science*, 52(3): 411-416.
- Williamson, S.L., J.J. Seibert, C.M. Glasier, R.E. Leithiser, J. Aronson, 1991. Ultrasound in advanced pediatric osteomyelitis. *Pediatric. Radiol.*, 21: 288-290.

Zhu, W.M., D.P. Wang, J.Y. Xiong, 2007. Biological characteristics and clinical application of scaffold materials for bone tissue engineering. *J. Clin. Rehab. Tissue. Eng. Res.*, 48: 23-26.

Zhu, W., J. Xiao, D. Wang, J. Liu, J. Xiong, L. Liu, X. Zhang, Y. Zeng, 2009. Experimental study of nano-HA artificial bone with different pore sizes for repairing the radial defect. *International Orthopaedics*, 33: 567-571.