Iranian Veterinary Surgery Association

IRANIAN JOURNAL OF VETERINARY SURGERY

Journal homepage: www.ivsajournals.com

ORIGINAL ARTICLE

Evaluation of Possible Beneficial Effect of Tricalcium Phosphate/Collagen (TCP/Collagen) Nanocomposite Scaffold on Bone Healing in Rabbits: Biochemical Assessments

Hanieh Farahi[1], Siamak Mashhadi-Rafie[1*], Alireza Jahandideh[2], Ahmad Asghari[2], Seyed Hamed Shirazi-Beheshtiha[3]

[1] Department of Small Animal Internal Medicine, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.

[2] Department of Veterinary Surgery, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.

[3] Department of Clinical Sciences, Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Iran.

Received: 10 July 2019
Accepted: 28 September 2019
Online: 6 October 2019

Keywords:
Reconstruction;
bone healing;
TCP/collagen nanocomposite;
Biochemistry;
Rabbit.

Abstract

Objective- The aim of this study was to evaluate possible beneficial effect of tricalcium phosphate/collagen (TCP/collagen) nanocomposite scaffold on bone healing in rabbits using biochemical assessments
Design- Experimental study
Animals- Eighteen healthy male white New Zealand rabbits
Procedures- The rabbits were marked with non-toxic color and randomly divided into two groups of 9 animals each. In the first group (Sham) the defect was made and with no treatment, the wound was closed. In the second group (TCP/C) the TCP/collagen nanocomposite scaffold was implanted into the defect. Before the procedures (day 0) and on 7, 15, 30, 45, and 60 postoperative days the blood samples were taken from the jugular vein and undergone hematological and biochemical assessments.
Results- The hematological, biochemical and oxidative stress parameters including WBC, RBC, HCT, PLT, neutrophil, lymphocytes, BUN, Creatinine, AST, ALT, ALP, SOD, GPX and MDA showed statistically significant differences between Sham and TCP/C groups (p<0.05).
Conclusion and clinical relevance- It was concluded that TCP/collagen nanocomposite improved the biochemical parameters in the nanocomposite treated animals and could be of clinical benefit in reconstruction of bone defects and could be considered as a scaffold in bone fractures.

* Correspondence to: Siamak Mashhadi-Rafie, Department of Clinical Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran. E-mail: sr1vet@yahoo.com

DOI: 10.22034/IVSA.2019.193769.1189

1. Introduction

Bone tissue, when injured, leads to dramatic changes in the quality of life of patients. It can limit the ability to perform basic tasks and frequently causes social and psychological problems.[1] Autografts are still considered the gold standard for bone repair. However, some complications may occur, such as bone nonunion and blood loss, which increases the need for blood transfusions.[2-4] Moreover, besides being an expensive procedure, there is a limited supply of tissue and it causes significant donor-site morbidity.[5,6]

Osteoblast transplantation using polymer scaffolds is a promising strategy that may overcome the limitations of traditional bone graft materials.[7] This strategy involves the use of polymer-cell constructs composed of either marrow stromal cells or calvarial cells that have been seeded into porous biodegradable polymer scaffolds. Osteogenic cells can be obtained from the host and grown in an appropriate carrier in vitro. Subsequently, the osteogenic tissue scaffold constructs can be grafted back into the host to regenerate bone. The transplanted cells may secrete new matrix as well as the factors necessary for bone tissue growth and ingrowth while the polymer matrix gradually degrades.[8]

One of the main goals of bone tissue engineering is the design of a biodegradable porous material scaffold integrated with biological cells and molecular cues able to guide the process of de novo tissue regeneration.[9,10] Biodegradable scaffolds are generally considered as indispensable elements for engineering living tissues. An ideal scaffold to be used for bone tissue engineering should possess characteristics of excellent biocompatibility, adequate pore size, controllable biodegradability and suitable mechanical properties.[11]

Tricalcium phosphate (TCP) is a tertiary calcium phosphate also known as bone ash. It serves as a rich source for calcium and phosphorus, which can be easily assimilated and absorbed. Beta-TCP is highly biocompatible and creates a resorbable interlocking network within the defect site to promote healing.[12,13] Collagen is the most abundant protein in the extracellular matrix (ECM) and has been considered to be a group of proteins with a characteristic molecular structure fibrillar structure, which contributes to the extracellular scaffolding.[14] That is to say, collagen plays an important role in maintaining the biological and structural integrity of ECM and provides physical support to tissues. Collagen possesses extensive sources as it is the main structural protein of most hard and soft tissues.[15] In addition, collagen offers low immunogenicity, a porous structure, permeability, good biocompatibility, and biodegradability and has functions to regulate the morphology, adhesion, migration, and differentiation of cells.[16,17] Nanocomposite biomaterials or bio-nanocomposites offer versatility in designing specific properties due to better control of interactions between nanoparticles and polymers.[18] Polymer nanocomposite biomaterials possess superior mechanical properties when compared with their macro- and microcomposite counterparts.[19]

In the present study, a critical segmental defect of rabbit femur was repaired using tricalcium phosphate/collagen (TCP/collagen) nanocomposite scaffold and the effects were assessed biochemically.

2. Materials and Methods

Preparation and characterization of tricalcium phosphate/collagen nanocomposite scaffold

The nanocomposite scaffold was prepared based on a method described by others.[20] In brief, collagen suspension was prepared in an aqueous alkali solution (pH = 12) at room temperature. Then TCP powder was slowly added into the collagen suspension (2:1) while stirring, after a homogenous suspension was formed, the glutaraldehyde solution was added as a cross-linking agent. The mixture froze in a refrigerator for 5 h at -40 °C. Porous composites were obtained after further lyophilization. The particle size of the tricalcium phosphate powder and the structure of the

TCP/collagen nanocomposite scaffold were determined using transmission electron microscope and scanning electron microscope techniques, respectively (Figure 1).

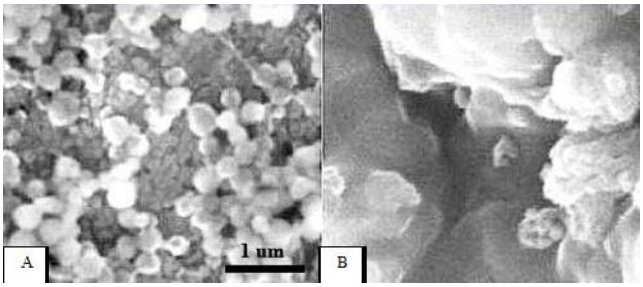

Figure 1. (A) TEM image of the calcium nanoparticles. (B) SEM image of the nanocomposite.

Study design and animals

Eighteen mature male New Zealand white rabbits, 6–8 months and with an approximate weight of 3–3.5 kg were included in the study. All animals were obtained from the same source and used in this study in order to decrease the genetic variability. The animals were housed separately (one rabbit per cage) and maintained on a standard pellet diet and tap water. Animal houses were in standard environmental conditions at a temperature of 18 ± 3 °C, the humidity of 60 ± 5% and natural light/dark cycle. Lateral femoral osteotomies were performed surgically. This investigation was approved by the institutional animal care and use committee (IACUC) at Islamic Azad University. Rabbits were randomized into two experimental groups of 9 animals each.

Surgery and animal grouping

Surgical procedures were done after an intramuscular injection of ketamine 10% (50 mg/kg), xylazine 5% (Rompun, 5 mg/kg).[7] The surgical site was shaven and the skin was cleaned with iodinated surgical soap. Aseptic technique was used throughout the surgical procedure. An incision of approximately 5 cm long was made along the medial right upper hind limb, and the mid diaphyseal surface of the femur was surgically exposed by blunt dissection. The periosteum was stripped from the bone using a periosteal elevator and an approximately a 6mm diameter – 5mm cylinder bilateral bone defect was created in the femur of one of the hind limbs. This osteotomy site was then irrigated with 0.9% saline, but periosteum around the osteotomy site was preserved and retracted with the overlying muscles. The osteotomy site was then treated according to the treatment protocol for each rabbit. After making the bone defects, all rabbits were marked with non-toxic color and randomly divided into two groups of 6 animals each. In the first group (Sham) the defect was made and with no treatment, the wound was closed. In the second group (TCP/C) the TCP/collagen nanocomposite scaffold was implanted into the defect (Figure 2).

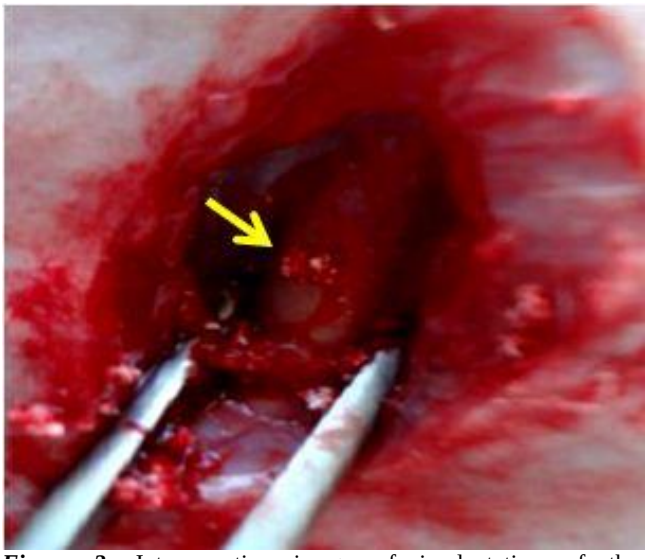

Figure 2. Intraoperative image of implantation of the nanocomposite into the created defect in the femur shaft.

The periosteum and subcutaneous tissues were then closed using single interrupted suture with Dexon 0/3 (Supa Medical Devices, Tehran, Iran). Antibiotics (penicillin G procaine 40,000 IU/kg IM, bid) (Nasr, Mashhad, Iran), dexamethasone (0.6 mg/kg, IM) (Abureihan, Tehran, Iran) and tramadol hydrochloride (5 mg/kg, IM, bid) (Abureihan, Tehran, Iran) were administered for three post-operative days.[7] Experimental animals were kept in separate cages to prevent self-injury. After the procedure, daily observation was performed and evidence of infection or other abnormalities were noted. Before the procedures (day 0) and on 7, 15, 30, 45 and 60 postoperative days the blood samples were taken from the jugular vein.

Biochemical studies

The blood samples were taken into EDTA-contained test tubes. The hematological parameters including white blood cell count (WBC), red blood cell count (RBC), hematocrit (HCT), platelet (PLT), neutrophil and lymphocytes were measured using the Exigo-vet analyzer (Stockholm, Sweden).

The blood samples were taken into EDTA contained test tubes for assessment of serum biochemical factors and left in room temperature. The samples were then centrifuged at 4000 rpm for 10 min and kept at -20 °C until assessments. The biochemical parameters including BUN, creatinine, Aspartate transaminase (AST), Alkaline phosphatase (ALP), and Alanine transaminase (ALT) were assessed using commercial kits (Dialab Kit, DIALAB GmbH, Neudorf Austria) by an automatic chemistry analyzer (BT1500, Rome, Italy). The oxidative stress parameters including malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPX) were assessed using Zell Bio GmbH (Germany) kit and evaluated by BIOTEK EL x800 ELISA plate reader.

Statistical analysis

For determining of normality of data, the one-sample Kolmogorov–Smirnov test was adopted. Results were analyzed using a factorial ANOVA with two between-subjects factors. Statistical analyses were performed in SPSS statistical software program for Windows (version 22.0, SPSS Inc., Chicago, USA). Statistically significant level was set at p <0.05.

3. Results

The hematological parameters including WBC count, RBC count, HCT, PLT, neutrophil and lymphocytes count showed statistically significant differences between Sham and TCP/C groups (p<0.05) (Figure 3)

The biochemical parameters including BUN, creatinine, AST, ALT, and ALP showed statistically significant differences between Sham and TCP/C groups (p<0.05) (Figure 4).

Oxidative stress parameters including MDA, SOD, and GPX showed statistically significant differences between Sham and TCP/C groups (p<0.05) (Figure 5).

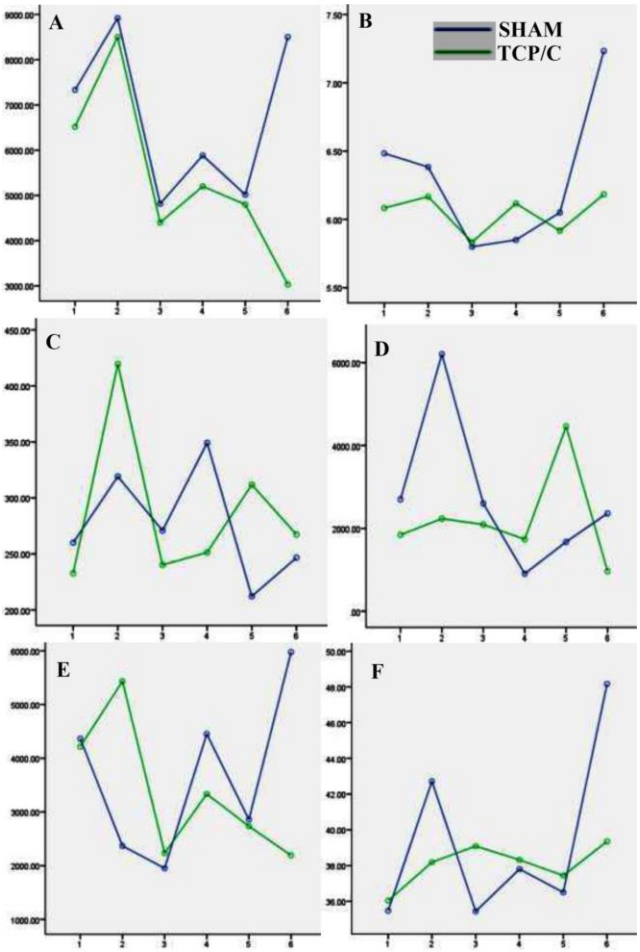

Figure 3. The hematological parameters. (A) white blood cell count (WBC), (B) red blood cell count (RBC), (C) hematocrit (HCT), (D) platelet (PLT), (E) neutrophil and (F) lymphocytes values. The numbers 1 to 6 represent days 0, 7,15,30,45 and 60 post- operation, respectively.

4. Discussion

The main goal of the present study was to assess bone promotion in surgical defects created in the femur of normal and experimental rabbits using TCP/collagen nanocomposite scaffold. The unique properties of nanocomposites have attracted notable research interest, especially in bioapplications owing to their structural features. The different types of nanocomposites including

organic- inorganic, inorganic -inorganic and bioinorganic nanomaterials have allowed their use in biomedical fields such as drug delivery and regenerative medicine. A nanocomposite is defined as a multiphase solid material in which one of the phases has one, two, or three dimensions less than 100 nm.[21] Aiming to replace traditional technologies in bone fracture treatment, such as grafting and metallic prostheses, the use of biocompatible and biodegradable polymers in bone tissue engineering was developed.[22]

The bone is one of the most rigid tissues in the human body, and is responsible for protecting and providing support to many organs, as well as playing a key role in mobility. Fractures of larger proportion are not able to spontaneously self-regenerate and require external

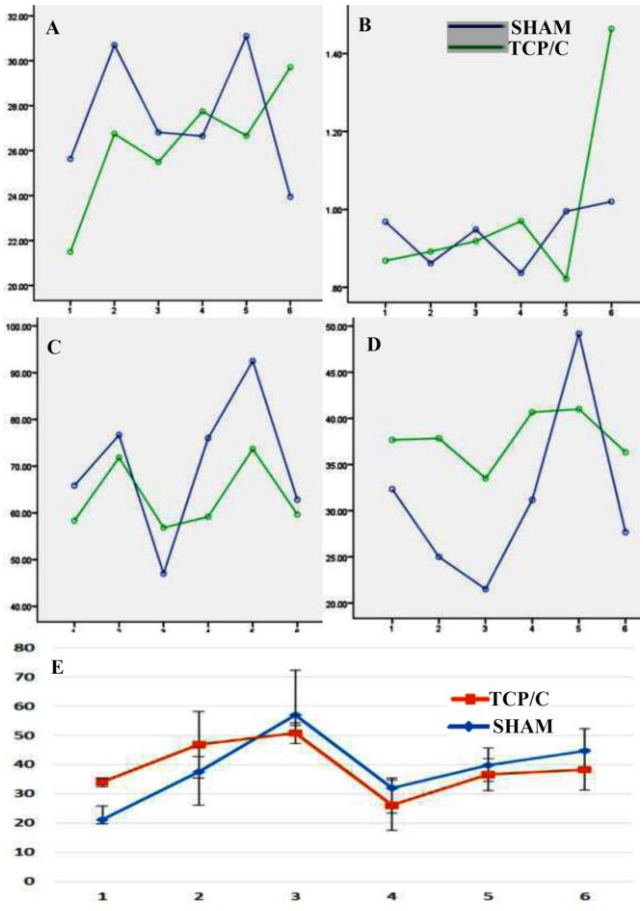

Figure 4: The biochemical parameters. (A) BUN, (B) creatinine, (C) Aspartate transaminase (AST), (D) Alanine transaminase (ALT) and (E) Alkaline phosphatase (ALP)values. The numbers 1 to 6 represent days 0, 7,15,30,45 and 60 post- operation, respectively.

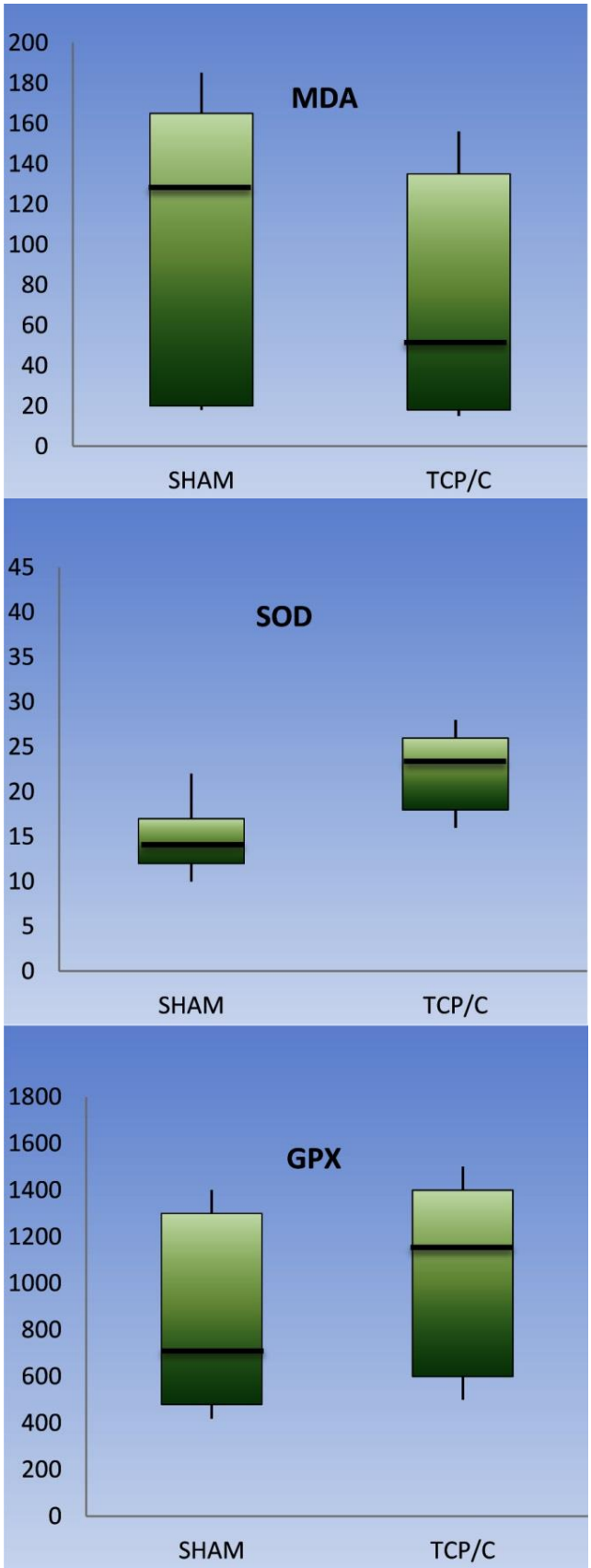

Figure 5. Box plot representing oxidative stress parameters including malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPX) in the experimental groups.

intervention, which is needed more and more as people grow older and there is a decrease in bone deposition activity. Hence, the need for bone tissue regeneration technology grows dramatically as the world population ages and the life expectancy rates increases.[23] Autografting has osteoconductive, osteoinductive and osteogenic properties, which allows for fast and complete integration without rejection due to the genic similarity of the grafted tissue. However, one of the problems with the use of this technology is the need to work with two surgical sites, which makes post-op recovery more difficult.[24] Allografting and xenografting are considered an alternative to the discomfort of two surgeries, but these types of grafting are extremely immunogenic, which increases the chances of failure as the initial osteoinductive phase can be destroyed by the immunological system and the inflammatory cells, causing necrosis of the osteoconductive cells. In order to avoid this adversity, synthetic bone grafts started being used, which means the creation of scaffolds that can temporarily stimulate and support bone regeneration, acting as artificial extracellular matrices that intent to stimulate bone repair.[25,26]

The ideal scaffold must be biocompatible, biodegradable and bioabsorbable, so it can degrade at the same rate as the neoformation of the bone, so the newly formed tissue is able to replace it. As the scaffold degrades, it loses its mechanical strength, however, if its degradation is controlled and takes a long time to happen, the mechanical properties are gradually transferred from the scaffold to the newly formed bone tissue.[22]

Calcium phosphates has been taken special attention, due to its excellent biocompatibility along with osteoconductive and osteoinductive properties.[27] Depending on its composition, particle size, and production process, it bears various degrees of bioactivity, which is the ability to chemically bond and be integrated into living bone through the formation of hydroxyl apatite.[28] In contrary, it bears low mechanical stability, making it unsuitable for load bearing applications.[29]

Furthermore, calcium phosphate is among newer generations of bone substitutes, with potential clinical applications in orthopaedics. Calcium phosphate granules have become materials of choice for bone repair because of its biocompatibility and osteoconductivity.[30] Calcium phosphate cement (CPC) is also an excellent bone grafting material, with good biocompatibility and a high modeling capacity to fill any bone-defect shape without leaving a gap.[31]

Collagen is the main fibrous structural protein in the bodies of living organisms, and a collagen scaffold is beneficial to cells grown *in vivo*.[32] The collagen-based scaffolds have been proven to possess excellent biocompatibility and sufficient mechanical properties and have gained great achievements in tissue engineering.[33] Fabricated collagen-calcium phosphate scaffolds using low- temperature 3D printing has been already used. The cytocompatibility and mechanical strength of scaffolds were maximized by tailoring a certain concentration of the phosphoric acid-based blind solution. Then, the scaffolds were implanted into a critically sized murine femoral defect for 9 weeks. Results indicated that the scaffolds were osteoconductive, and the scaffolds were partly broken down with new bone forming.[34]

Healing of segmental bone defects with cortical bone after implantation of the complex of b-TCP granules and 5% collagen with rhFGF-2 has also been reported.[35] Others developed a biodegradable sponge composite for bone tissue engineering by combining bTCP and collagen. In addition, they sought to determine the optimal bTCP granules/collagen ratio by evaluating and bone formation *in vivo*.[36]

Nanoparticles of hydroxyl apatite combined with synthetic biodegradable polymers have been developed to produce nanocomposites for bone tissue engineering.[37-40] Others reported that scaffold materials used for bone formation should bear the following properties: The scaffold material must allow osteoblast attachment, since these anchorage-dependent cells require a supportive matrix to survive. It

must provide an appropriate environment for osteoblast proliferation and function. It should allow ingrowth of vascular tissue to ensure the survival of transplanted cells. The material should be biodegradable and its degradation products should be easily metabolized and excreted. It should be able to be processed into irregular three-dimensional shapes.[41]

Augmented levels of oxidative stress which is induced by nanocomposite lead to rejection of implants and as the result of defective defense mechanisms at lower levels of oxidative stress, the infection is expected. Hence, to combat undesired consequences, keeping the oxidative stress at a physiologic level is crucial.[42] Understanding of the cell- material interaction in regard to oxidative stress levels is required for fabrication of novel nanocomposite.[43] Application of TCP-Collagen nanocomposite in the humorous bone defects has already been proposed in rabbits.[44] The main purpose of the present study was to demonstrate the safety of the nanocomposite based on biochemical factors and the possibility of oxidative stress by TCP-Collagen nanocomposite. In a bone fracture, the ROS levels significantly are increased, which is originated from the damaged tissue. Due to osteoclastic activity, serum MDA level will also increase that in turn will lead to the utilization of serum MDA.[45] The activity of antioxidant enzymes such as SOD and GPX is insufficient to keep pace with osteoclastic superoxidation, hence and as a result of increased levels of serum MDA, the defense mechanism will be compromised.[45] Excessive reactive oxygen species (ROS) production overwhelms antioxidant defense mechanisms which results in loss of the fundamental balance between ROS production and antioxidant defense mechanisms.[46] Assessment of SOD, GPX, and CAT has shown increased lipid peroxidation and decreased antioxidant enzymes in tissues that were in contact with ceramic and titanium.[47] Others reported that antioxidant effects of agarose-chitosan bone graft substitute increase in GPX, SOD, and CAT in the bone tissue 30 days after application. MDA level was also found to increase in the treatment group.[48] GPX also acts as a detoxifier in lipid peroxidation, while SOD causes superoxide ($-O_2$) to decompose into molecular oxygen (O_2) or hydrogen peroxide (H_2O_2).[49] In the present study, the MDA level was increased on days 15 and 30 post-surgery in the TCP/C group and it was reduced on days 45 and 60. The MDA levels in the SHAM group were significantly more than those of the TCP/C group indicating more enhanced osteoclast activity. GPX enzyme level was increased on days 15 and 30 in the TCP/C group. The enzyme is responsible for lipid peroxide detoxification and together with CAT acts for H_2O_2 detoxification. Thus, when the MDA levels are high, GPX begins to increase as well. SOD enzyme levels were slightly increased on the day 15 after surgery. SODs are a group of enzymes that catalyze the dismutation of superoxide radicals to molecular oxygen and H_2O_2. Blood chemistry profiles of the present study were also evaluated for detection of possible abnormalities along with assessment of biocompatibility of nanomaterial. AST, ALT, and ALP, all indicators of liver damage as well as creatinine and urea levels, reflecting renal function, were all within the normal range in all groups. The upsurge observed in ALP after surgery could be due to an increase in bone- specific alkaline phosphatase isoenzyme, which is the consequence of increased osteoblast activity in reconstruction phase.[50] In the present study, the assessed hematological parameters were within the normal limit and the WBC count indicated no sign of any systemic inflammatory reaction.

In conclusion, the findings of the present study demonstrated the developed nanocomposite did not triggered tissue inflammation and organ damage and improved the biochemical parameters in the nanocomposite treated animals and could be of clinical benefit in the reconstruction of bone defects and could be considered as a scaffold in bone fractures.

Acknowledgement

This work was a part of a dissertation of first author (H.F.)

submitted as a Partial Fulfillment of the Degree of PhD. The author is thankful to Deputy for Research of the University for their support.

Conflict of Interests

None.

References

1. Costa-Pinto AR, Reis RL, Neves NM. Scaffolds based bone tissue engineering: the role of chitosan. *Tissue Engineering and Regenerative Medicine Part B*, 2011; 17: 331-347.
2. Muramatsu K, Doi K, Ihara K, Shigetomi M, Kawai S. Recalcitrant posttraumatic nonunion of the humerus: 23 patients reconstructed with vascularized bone graft. *Acta Orthopaedica Scandinavica*, 2003; 74: 95-97.
3. Al-Sayyad, M, Abdulmajeed T. Fracture of the anterior iliac crest following autogenous bone grafting. *Saudi Medical Journal*, 2006: 27: 254-258.
4. Pritsch T, Bickels J, Wu C-C, Squires HM, Malawer MM. The risk for fractures after curettage and cryosurgery around the knee. *Clinical Orthopaedics and Related Research*, 2007; 458:159-167.
5. Chen Y-C, Chen C-H, Chen P-L, Huang I-Y, Shen Y-S, Chen C-M. Donor site morbidity after harvesting of proximal tibia bone. *Head and Neck*. 2006; 28:496-500.
6. Chou LB, Mann RA, Coughlin MJ, McPeake WT, Mizel MS. Stress fracture as a complication of autogenous bone graft harvest from the distal tibia. *Foot and Ankle International* 2007; 28:199-201.
7. Vinatier C, Gauthier O, Masson M, Malard O, Moreau A, Fellah BH, Bilban M, Spaethe R, Daculsi G, Guicheux JJ. Nasal chondrocytes and fibrin sealant for cartilage tissue engineering. *Journal of Biomedical Materials Research Part A*, 2009; 89(1): 176-85.
8. He X, Kawazoe N, Chen G. Preparation of cylinder-shaped porous sponges of poly (L-lactic acid), poly (DL-lactic-co-glycolic acid), and poly (ε-caprolactone). *BioMed Research International*, 2014; 106082.
9. Qingchun Z, Ke T, Zhaoyang Y, Yan Z, Wensong T, Meidong L. Preparation of open porous polycaprolactone microspheres and their applications as effective cell carriers in hydrogel system. *Materials Science and Engineering C*, 2012; 32: 2589–2595.
10. Salerno A, Zeppetelli S, Di Maio E, Iannace S, Netti PA. Architecture and properties of bi-modal porous scaffolds for bone regeneration prepared via supercritical CO_2 foaming and porogen leaching combined process. *Journal of Supercritical Fluids*, 2012; 67: 114–122.
11. Yang SF, Leong KF, Du ZH, Chua CK. The design of scaffolds for use in tissue engineering. Part I. Traditional factors. *Tissue Engineering*, 2001; 7: 679.
12. Ng A, Saim AB, Tan KK, Tan GH, Mokhtar SA, Rose IM, Othman F, Idrus RB. Comparison of bioengineered human bone construct from four sources of osteogenic cells. *Journal of Orthopaedic Science*, 2005; 10: 192–199.
13. Tan KK, Shamsui BS, Chua KH, Chua KH, Ng MH, Ruszymah BH, Aminuddin BS, Loqman MY. Bone graft substitute hydroxyapatite scaffold seeded with tissue engineered autologous osteoprogenitor cells in spinal fusion: early result in sheep as a model. *Medical Journal of Malaysia*, 2005; 60: 53–58.
14. Gelse K, P€oschl E, Aigner T. Collagens – structure, function, and biosynthesis. *Advanced Drug Delivery Reviews*, 2003; 55: 1531–1546.
15. Kuivaniemi H, Tromp G. Type III collagen (COL3A1): Gene and protein structure, tissue distribution, and associated diseases. *Gene*, 2019; 707: 151-171.
16. Chevallay B, Herbage D. Collagen-based biomaterials as 3D scaffold for cell cultures: applications for tissue engineering and gene therapy. *Biological Engineering and Computing*, 2000; 38: 211–218.
17. Wolf K, Alexander S, Schacht V, Coussens LM, von Andrian UH, van Rheenen J, Deryugina E, Friedl P. Collagen-based cell migration models in vitro and in vivo. *Seminars in Cell and Developmental Biology*, 2009; 20: 931–941.
18. Hule RA, Pochan DJ. Polymer nanocomposites for biomedical applications. *MRS Bulletin*, 2007; 32: 354–358.
19. Winey KI, Vaia RA. Polymer nanocomposites. *MRS Buletin*, 2007; 32: 314–322.
20. Mohseni M, Jahandideh A, Abedi G, Akbarzadeh A, Hesaraki S. Assessment of tricalcium phosphate/collagen (TCP/collagen) nanocomposite scaffold compared with hydroxyapatite (HA) on healing of segmental femur bone defect in rabbits. *Artificial Cells, Nanomedicine, and Biotechnology*, 2018; 46(2): 242-249.

21. Fajardo AR, Pereira AGB, Muniz EC. Hydrogels nanocomposites based on crystals, whiskers and fibrils derived from biopolymers. *Civil and Structural Engineering Materials Science*, 2015; 74: 43–71.
22. Sheikh, Z., Najeeb, S., Khurshid, Z., Verma, V., Rashid, H. and Glogauer, M. Biodegradable Materials for Bone Repair and Tissue Engineering Applications. *Materials (Basel)*, 2015; 8: 5744-5794.
23. Wang W, Yeung KWK. Bioactive Materials Bone Grafts and Biomaterials Substitutes for Bone Defect Repair: A Review. *Bioactive Materials*, 2017; 1-24.
24. Roberts TT, Rosenbaum AJ, Roberts TT, Rosenbaum AJ. Bone Grafts, Bone Substitutes and Orthobiologics: The Bridge between Basic Science and Clinical Advancements in Fracture Healing. *Organogenesis*, 2012; 8: 114-124.
25. Goldberg VM, Akhavan S. Biology of Bone Grafts. In: Lieberman, JR, Friedlaender, GE, eds., Bone Regeneration and Repair, Humana Press, New York, 2005; 57-65.
26. Wydra FB. Allografts. *Clinics in Sports Medicine*. 2017; 36: 509-523.
27. Schumacher M, Uhl F, Detsch R, Deisinger U, Ziegler G. Static and dynamic cultivation of bone marrow stromal cells on biphasic calcium phosphate scaffolds derived from an indirect rapid prototyping technique. *Journal of Materials Science: Materials in Medicine*. 2010; 21: 3039–3048.
28. Yun HS, Park JW, Kim SH, Kim YJ, Jang JH. Effect of the pore structure of bioactive glass balls on biocompatibility in vitro and in vivo. *Acta Biomaterialia*, 2011; 7: 2651–2660.
29. Bhakta S, Pattanayak D, Takadama H, Kokubo T, Miller CA, Mirsaneh M, Reaney IM, Brook I, van Noort R, Hatton PV. Prediction of osteoconductive activity of modified potassium fluorrichterite glass-ceramics by immersion in simulated body fluid. *Journal of Materials Science: Materials in Medicine*, 2010; 21: 2979–2988.
30. Komath M, Varma HK, Sivakumar R. On the development of an apatitic calcium phosphate bone cement. *Bulletin of Materials Science*, 2000; 23: 135–140.
31. Bohner M, Baroud G. Injectability of calcium phosphate pastes. *Biomaterials*. 2005; 26:1553–1563.
32. Zuber M, Zia F, Zia KM, Tabasum S, Salman M, Sultan N. Collagen based polyurethanes-a review of recent advances and perspective. *International Journal of Biological Macromolecules*, 2015; 80: 366–374.
33. Liu S, Wu J, Liu X, Chen D, Bowlin GL, Cao L, Lu J, Li F, Mo X, Fan C. Osteochondral regeneration using an oriented nanofiber yarn-collagen type I/ hyaluronate hybrid/TCP biphasic scaffold. *Journal of Biomedical Materials Research Part A*, 2015; 103: 581–592.
34. Inzana JA, Olvera D, Fuller SM, Kelly JP, Graeve OA, Schwarz EM, Kates SL, Awad HA. 3D printing of composite calcium phosphate and collagen scaffolds for bone regeneration. *Biomaterials*, 2014; 35: 4026–4034.
35. Komaki H, Tanaka T, Chazono M, Kikuchi T. Repair of segmental bone defects in rabbit tibiae using a complex of beta-tricalcium phosphate, type I collagen, and fibroblast growth factor-2. *Biomaterials*, 2006; 27: 5118–5126.
36. Tomonori M, Tatsuo N, Koh-ichi K. Development of P-tricalcium phosphate/collagen sponge composite for bone regeneration. *Dental Materials Journal*, 2006; 25: 1138–1144.
37. Boissard CI, Bourban PE, Tami AE, Alini M, Eglin D. Nanohydroxyapatite/poly (ester urethane) scaffold for bone tissue engineering. *Acta Biomaterialia*, 2009; 5: 3316–3327.
38. Jack KS, Velayudhan S, Luckman P, Trau M, Grøndahl L, Cooper-White J. The fabrication and characterization of biodegradable HA/PHBV nanoparticle-polymer composite scaffolds. *Acta Biomaterialia*, 2009; 5: 2657–2667.
39. Jayabalan M, Shalumon KT, Mitha MK, Ganesan K, Epple M. Effect of hydroxyapatite on the biodegradation and biomechanical stability of polyester nanocomposites for orthopaedic applications. *Acta Biomaterialia*, 2010; 6: 763–775.
40. Asran ASH, Henning S, Michler GH. Polyvinyl alcohol-collagenhydroxyapatite biocomposite nanofibrous scaffold: mimicking the key features of natural bone at the nanoscale level. *Polymer*, 2010; 51: 868–876.
41. Ishaug-Riley SL, Crane-Kruger GM, Yaszemski MJ, Mikos AG. Bone formation by three dimensional stromal osteoblasts culture in biodegradable polymer scaffolds. *Journal of Biomedical Materials Research Part A*, 1997; 36: 17–28.
42. Mouthuy PA, Snelling SJB, Dakin SG, Milković L, Gašparović AČ, Carr AJ, Žarković N. Biocompatibility of implantable materials: An oxidative stress viewpoint. *Biomaterials*, 2016; 109: 55-68.
43. Pandey A, Midha S, Sharma RK, Maurya R, Nigam VK, Ghosh S, Balani K. Antioxidant and

antibacterial hydroxyapatite-based biocomposite for orthopedic applications. *Materials Science and Engineering C-Materials for Biological Applications*, 2018; 88: 13-24.

44. Palmer I, Nelson J, Schatton W, Dunne N, Buchanan F, Clarke SA. Biocompatibility of calcium phosphate bone cement with optimized mechanical properties. *Journal of Materials Science: Materials in Medicine*, 2016; 27(12): 191-199.
45. Bowles SA, Kurdy N, Davis AM, France MW, Marsh DR. Serum osteocalcin, total and bone-specific alkaline phosphatase following isolated tibial shaft fracture. Annals of *Clinical Biochemistr*, 1996; 33(Pt 3): 196-200.
46. Zinnuroglu M, Dincel AS, Kosova F, Sepici V, Karatas GK. Prospective evaluation of free radicals and antioxidant activity following 6-month risedronate treatment in patients with postmenopausal osteoporosis. *Rheumatology International*, 2012; 32(4): 875-880.
47. Ozmen I, Naziroglu M, Okutan R. Comparative study of antioxidant in tissues surrounding implant in rabbits. *Cell Biochemistry and Function*, 2006; 24(3): 275-281.
48. Jebahi S, Saleh GB, Besaleh S, Oudadesse H, Mhadbi M, Rebai T, Keskes H4, E Feki A. Genotoxicity effect, antioxidant and biomechanical correlation: experimental study of agarose-chitosan bone graft substitute in New Zealand white rabbit model. *Proceedings of the Institution of Mechanical Engineers, Part H*, 2014; 228(8): 800-809.
49. Khanna P, Ong C, Huat B, Baeg GH. Nanotoxicity: An interplay of oxidative stress, inflammation and cell death. *Nanomaterials (Basel)*, 2015; 30; 5(3): 1163-1180.
50. Paskalev D. Time course of serum malondialdehyde concentrations as a marker of oxidative stress in experimental canine osteotomies fixed by two different techniques. *Comparative Clinical Pathology*, 2009; 18(3): 265-268.

نشریه جراحی دامپزشکی ایران
سال ۲۰۱۹، جلد ۱۴ (شماره ۲)، شماره پیاپی ۳۱

چکیده

ارزیابی اثرات احتمالی مثبت داربست نانوکامپوزیت تری کلسیم فسفات/کلاژن بر روی ترمیم استخوان در خرگوش: سنجش بیوشیمیایی

هانیه فرهی[۱]، سیامک مشهدی رفیعی[۱]، علیرضا جهان‌دیده[۲] ،احمد اصغری[۲]، سیدحامد شیرازی بهشتی‌ها[۳]

[۱] گروه داخلی دام کوچک، دانشکده دامپزشکی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران.
[۲] گروه جراحی دامپزشکی، دانشکده دامپزشکی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران.
[۳] گروه علوم درمانگاهی، دانشکده دامپزشکی، واحد کرج، دانشگاه آزاد اسلامی، کرج، ایران.

هدف- هدف از این مطالعه ارزیابی احتمال اثرات مثبت داربست نانوکامپوزیت تری کلسیم فسفات/کلاژن بر روی ترمیم استخوان در خرگوش با استفاده از سنجش بیوشیمیایی بود.

طرح مطالعه- مطالعه تجربی

حیوانات- ۱۸ قطعه خرگوش سفید نر نیوزلندی سالم

روش کار- خرگوش ها با رنگ غیر سمی مشخص و به دو گروه ۹ تایی به طور تصادفی تقسیم شدند. در گروه اول (شم) نقیصه ای بدون مداخله درمانی در استخوان ایجاد شد و زخم بسته شد. در گروه دوم (TCP/C) داربست نانوکامپوزیت تری کلسیم فسفات/کلاژن در نقیصه کارگذاری شد. قبل از جراحی (روز صفر) و رئزهای ۷، ۱۵،۳۰،۴۵ و ۶۰ بعد از جراحی نمونه های خون وداجی اخذ و تحت مطالعات خونشناسی و بیوشیمیایی قرار گرفتند.

نتایج- پارامترهای خون شناسی، بیوشیمیایی و استرس اکسیداتیو شامل سلول های سفی و قرمز، هماتوکریت، پلاکت ها، انفوسیت ها، اوره، کراتینین، AST، ALT، ALP، SOD، GPX و MDA نشان دادند که تفاوت معنی داری آماری بین گروه ها وجود داشت ($p <$ ۰/۰۵).

نتیجه‌گیری و کاربرد بالینی- نتیجه گیری شد که داربست نانو کامپوزیت تری کلسیم فسفات/کلاژن پارامترهای بیوشیمیایی را در گروه درمان شده با نانوکامپوزیت را بهبود بخشیدند که میتواند در بازسازی نقیصه استخوانی از نظر کلینیک مفیدبوده و می توان آنرا به عنوان داربست در ترمیم شکستگی ها در نظر گرفت.

واژه‌های کلیدی- بازسازی، ترمیم استخوان، داربست نانو کامپوزیت تری کلسیم فسفات/کلاژن، بیوشیمی، خرگوش