J Oral Res
oral and craniofacial sciences

Article

The effect of the new hemostatic agent Ostene® on bone healing: An experimental study in rabbits.

Alyaa I. Naser.[1]

Affiliations: [1]Asst. lecturer. Department of Oral and Maxillofacial surgery, College of Dentistry, University of Mosul. Iraq.

Corresponding author: Alyaa I. Naser. Asst. lecturer. Department of Oral and Maxillofacial surgery, College of Dentistry, University of Mosul. Iraq. Phone: (964) 7701766626. E-mail: alyaaismael@uomosul.edu.iq

Receipt: 06/29/2018 Revised: 08/25/2018
Acceptance: 11/12/2018 Online: 11/13/2018

Abstract: Introduction: Ostene® is a water-soluble wax-like alkylene oxide copolymer preparation for use as a mechanical hemostatic agent. This study aims to evaluate the effects of Ostene® on bone healing. Materials and Methods: Twenty albino rabbits were divided into four groups according to post-treatment follow-up (24 hr, 3 days, 7 days, 14 days) with five rabbits in each group. Each rabbit in all groups was treated with two study materials (Ostene® and Gelfoam®). Three holes were made in the mandibular bone of each rabbit using 5mm surgical bur; two holes were made on right side: one for testing Ostene® and another for Gelfoam®. A third hole, on the left side of mandible, was not treated, and was used as a control. Finally, the incision was closed. The specimens were collected at different days post-treatment and examined by histopathology. Result and Discussion: This study showed that there is a significant difference (p-value≤ 0.05) between the Ostene® group and the other groups (Gelfoam® and control). At 24 hr post intervention, there is a significant difference in osteoblast cell formation (p-value=0.03), and osteoclast cell formation (p-value=0.05). New blood vessel formation, osteoblast and osteoclast cell formation for Ostene® group at 3 days post-intervention were also significantly different (p-values = 0.05, 0.03, 0.04, respectively). At 7 days post-intervention p-values were 0.05 for osteoblast formation and 0.04 for osteoclast formation, respectively. After 14 days of healing p-value for osteoblast cell formation in the Ostene® group was 0.05 and 0.04 for osteoclast cell formation. Conclusions: The bone hemostatic agent Ostene® is an effective at enhancing osteogenesis by initiating proliferation of osteoblast and osteoclast cells.

Keywords: Bone hemostatic agent; ostene; alkylene oxide copolymer; water soluble wax; wound healing.

Conflict of interests: The author declare no conflict of interests.

Ethics approval: Institutional ethical committee

Funding: The research is self-funded; there is no financial conflict with another party.

Authors' contributions: The author was solely responsible for conducting the whole study.

Acknowledgements: Great thanks to pathologist Asst. Prof. Dr. Manar Mudhafar, Ph.D. Oral Pathology for her efforts and support.

Cite as: Naser AI. The effect of the new hemostatic agent Ostene® and bone hea-ling: An experimental study in rabbits. J Oral Res 2018;7(8):350-355.
doi:10.17126/joralres.2018.075

INTRODUCTION.

Bone is a complex, highly organized and specialized connective tissue. It is a unique tissue that is healed by regeneration rather than repair. Bone healing consists of three overlapping phases. The first one is an inflammation process that starts immediately after trauma. There is an invasion of inflammatory cells, hematoma formation, and mesenchymal cells migration occurs. Granulation tissue formation is initiated by fibroblasts and mononuclear cells, which then matures to connective tissue that later converts to cartilage. Cartilage becomes woven bone by a process of mineralization, is then replaced by cortical bone and the medullary cavity is restored. After that, the remodeling process of the bone is started which is a slow process that occurs over months and years; adequate bone strength is typically achieved in 3-6

ISSN Online 0719-2479 - www.joralres.com © 2018

months.[1-4] During surgery that involves bone, bleeding can be controlled with suitable hemostatic agents. The choice of a suitable hemostatic agent and the time of its application require full consideration of their mechanisms of action, efficacy and possible adverse side effects.[5] Currently, many hemostatic agents are available; Ostene® is one of the most effective and easy to use material. Ostene® is an alkylene oxide copolymer wax-like preparation, a kind of water-soluble wax (WSW), and cranial and spinal surgeries were the first to use it.[6] Many studies have shown that Ostene® is a biocompatible and absorbable hemostatic agent. It induces immediate hemostasis by creating a mechanical and physical barrier but does not act at a biochemical level.[7,8] In 2001 Wang *et al.,*[8] firstl described the use of Ostene® as alternative to bone wax. The authors found that Ostene® did not inhibit bone growth, while achieving hemostasis. In other studies it was found that Ostene® is a hydrophilic and water soluble material, it does not interfere with coagulation and it is considered a bio-inert material. It is eliminated from the body unchanged through renal clearance within 48 hr,[9,10] but it is an expensive.[11] It is approved by the Food and Drug Administration as a water soluble implant material indicated for use in the control of bleeding of bone surfaces.[12] Gelfoam® is a water-insoluble, off-white, non-elastic, porous, malleable hemostatic agent prepared from purified porcine skin gelatin. It can absorb up to 45 times its weight of blood.[13] The hemostatic effect of Gelfoam® is a physical process rather than having a direct effect on the clotting cascade.[14] Gelfoam® has the ability of swelling more than collagen or cellulose and can double its volume, and although this swelling provides a good mechanical hemostatic action it is considered a negative characteristic, particularly when used near nerves as it causes compressive problems. Gelatin foam is absorbed within 4 to 6 weeks, and although it is derivative of animal products, it is non-antigenic.[15] Gelfoam® can be applied either as a dry sponge, moistened with sodium chloride solution, or saturated with topical purified thrombin.[16] The drawbacks of using gelatin include an increased rate of infection, granuloma formation, fibrosis, and the potential for breaking the clot if the sponge is removed.[17]

MATERIALS AND METHODS.

This work has been approved by the appropriate ethical committees related to the institution in which it was performed, and animal care was in accordance with institution guidelines. Ostene® (alkylene oxide copolymer) was provided by Ceremed Inc., and Gelfoam® was purchased from SEPTODONT.

Experimental Model: Twenty 6-8 months old male albino rabbits were used, each weighing about 2.3±0.5kg. The animals were housed in an animal facility organized for that purpose, fed a normal diet and water ad libitum. Each individual animal was given an intramuscular dose of ketamine hydrochloride 4mg/kg in 50mg/ml and xylazine base 5mg/kg in 20mg/ml,[18] injected into the thigh muscle. After 10-15 minutes, anesthetic integrity was checked by testing loss of the ear pinch reflex.[19]

Experimental groups

Twenty albino rabbits were divided into four groups according to sacrificing days post-intervention, corresponding to follow-up periods (24 hr, 3 days, 7 days, 14 days), with five rabbits per group. Every rabbit in all groups was treated with both study materials (Ostene® and Gelfoam). Three holes were made in the mandibular bone of each rabbit using a 5mm surgical bur; two holes were made on the right side: one for testing Ostene® and another for Gelfoam®. A third hole, on the left side of mandible, was not treated, and was used as a control.

Group 1: 24 hr, Ostene, Gelfoam, and no material control.
Group 2: 3 days, Ostene, Gelfoam, and no material control.
Group 3: 7 days, Ostene, Gelfoam, and no material control.
Group 4: 14 days, Ostene, Gelfoam, and no material control.

Surgical procedure and post-operative care

With aseptic surgical technique the animals underwent submandibular incision, the surgical site was exposed (mandibular bone), then two holes were made on the right side and a third hole on left side of mandible using a 5mm surgical bur, and appropriate study materials were applied. Finally the incision was closed using VICRYL® coated polyglactin suture 3-0. A single dose of 5mg/kg of the antibiotic oxytetracycline was injected intramuscular immediately post operatively.[20] The operated animals were separately caged until full recovery from anesthesia. The operated animals were closely observed during first twenty four hours post-operatively, particularly regarding their

feeding and physical activity. All animals began their normal activities and eating within 3-4 hr postoperatively.

After completing the given healing period, the animals were sacrificed and the mandible was dissected and stored in 10% formalin for fixation. The mandible of each group was divided into three pieces according to the material used (Ostene®, gelfoam and control). The bone specimens were then decalcified in 10% formic acid and 10% hydrochloric acid. After decalcification, the specimens were dehydrated through graded series of ethanol and xylene (70-99%), then embedded in paraffin wax, sectioned by microtome (4µm thickness) and stained using hematoxylin and eosin.

Histological Criteria

To assess the speed of healing, we used criteria according Lucaciu *et al.*,[21] with some modifications as follows:

A. Amount of granulation tissue

1. Profound | 2. Moderate | 3. Scant | 4. Absent

B. Amount of inflammatory infiltration

1. Plenty | 2. Moderate | 3. Few | 4. Absent

C. Neo-formation of blood vessel

0. Absent | 1. Present at peripherally | 2. Present at centrally| 3. Present at peripherally and centrally

D. Presence of osteoblast

0. Absent | 1.Present at peripherally | 2.Present at centrally| 3.Present at peripherally and centrally

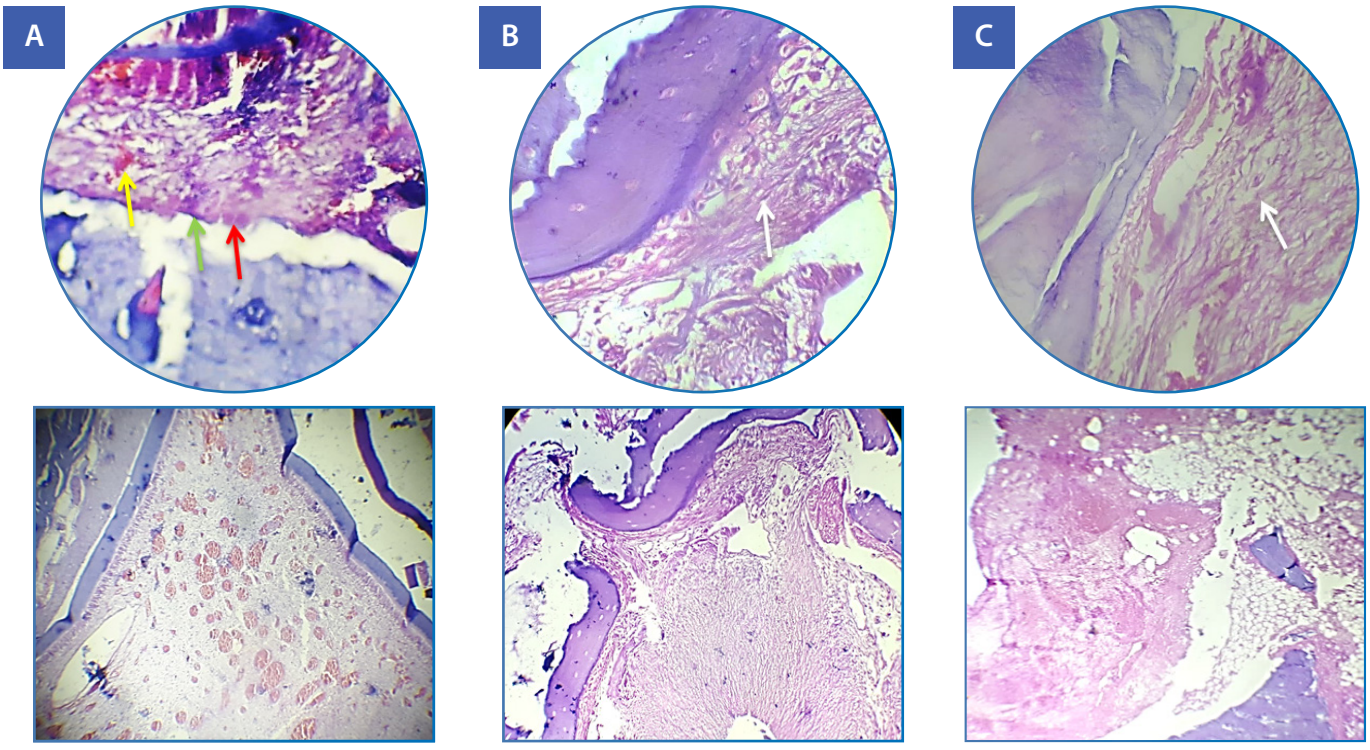
E. Presence of osteoclast

0. Absent | 1. Present at peripherally | 2. Present at centrally| 3. Present at peripherally and centrally

Histopathological examinations were done by using light microscope, Olympus (at X40, X10,) and read by two expert histopathological examiners separately. The final score was the mean values of the two readings.

Statistical Analysis: The data were processed statistically using the SPSS version 21 for Windows 10 pro, Lenovo laptop think pad L460. The associations between variables for histological analysis were analyzed using Friedman NPar Test for comparing the differences between groups within same period, Wilcoxon Signed-Rank Test for determining the group that led to differences within the same period.

Figure 1. Histological analysis of bone healing in mandible defects. Representative images at 3 days after surgery. H&E staining demonstrates osteoblasts (red arrow), and osteoclast (green arrow), and new blood vessel (yellow arrow) in the alkylene oxide copolymer (Ostene)-treated defects.



A. With mostly fibrous tissue (white arrows) observed at the site of the gelfoam. **B.** Control treated defects. **C.** Low magnification (x10, left) and high magnification (x40, right) are shown.

ISSN Online 0719-2479 - www.joralres.com © 2018

Table 1. Mean rank and *p*-values of Friedman Test for histopathological readings.

Periods	Variables	Mean rank and p-values of Friedman Test for Histopathological readings				
		Inflammation	Granulation tissue	new Blood vessel formation	formation Osteoblast	Osteoclast formation
24 hrs	Ostene	1.20	1.80	2.80	2.60	2.20
	Gelfoam	2.30	1.40	2.00	2.20	2.20
	Control	2.50	2.80	1.20	1.20	1.60
	p-values	0.038*	0.039*	0.041*	0.047*	0.449
3 days	Ostene	1.20	1.60	2.40	2.90	2.70
	Gelfoam	2.10	2.20	2.40	1.70	1.80
	Control	2.70	2.20	1.20	1.40	1.50
	p-values	0.042*	0.135	0.032*	0.015*	0.039*
7 days	Ostene	1.90	1.80	2.40	2.70	2.70
	Gelfoam	2.20	1.80	2.20	2.10	2.10
	Control	1.90	2.40	1.40	1.20	1.20
	p-values	0.368	0.264	0.211	0.022*	0.035*
14 days	Ostene	3.00	2.00	2.10	2.30	2.80
	Gelfoam	1.50	2.00	2.20	2.60	2.10
	Control	1.50	2.00	1.70	1.10	1.10
	p-values	0.007*	.	0.678	0.025*	0.014*

*statistical significant result p≤ 0.05

Table 2. Wilcoxon signed-rank test for histopathological readings between variables within same period.

Periods	Histopathological Readings	Wilcoxon signed rank-test for histopathological readings between variables within same period		
		Variables		
		Ostene-gelfoam	Ostene-control	Gelfoam-control
24 hr	Inflammation	0.06	0.06	0.2
	Granulation tissue	0.2	0.1	0.03*
	New blood vessel formation	0.1	0.03*	0.1
	Osteoblast formation	0.2	0.05*	0.04*
	Osteoclast formation		*p*-value = 0.4 in Friedman test	
3 days	Inflammation	0.05*	0.04*	0.07
	Granulation tissue		*p*-value = 0.1 in Friedman test	
	New Blood vessel formation	1.00	0.05*	0.06
	Osteoblast formation	0.05*	0.03*	0.3
	Osteoclast formation	0.08	0.04*	0.3
7 days	Inflammation		*p*-value = 0.3 in Friedman test	
	Granulation tissue		*p*-value = 0.2 in Friedman test	
	New Blood vessel formation		*p*-value = 0.2 in Friedman test	
	Osteoblast formation	0.1	0.05*	0.05*
	Osteoclast formation	0.4	0.04*	0.1
14 days	Inflammation	0.03*	0.03*	1.00
	Granulation tissue		*p*-value =(.) in Friedman test	
	New Blood vessel formation		*p*-value = 0.6 in Friedman test	
	Osteoblast formation	1.00	0.05*	0.03*
	Osteoclast formation	0.1	0.04*	0.05*

*statistical significant result p≤ 0.05

RESULTS.

The results of the analysis of the differences between the two study materials and control regarding inflammation, granulation tissue formation, blood vessel formation, and osteoclast and osteoblast formation, at given time periods of healing are shown in Table 1.

Wilcoxon signed-rank test revealed the differences between ostene-gelfoam, ostene-control, and gelfoam-control for all periods of healing as shown in Table 2. Statistical significance was set at p-value ≤0.05

DISCUSSION.

Bleeding from the defects treated with Ostene® completely ceased shortly after its application compared to the defects treated with gelfoam and control. This observation is in agreement with other studies, wehre it was found that Ostene® formed a mechanical barrier that prevents the flow of blood from the exposed bone.[9,22] Handling of Ostene® material compared to gelfoam was more favorable, as it was stuck more to the bone. Furthermore, Ostene could be better reshaped to match the size of defect. This study was designed to histopathologicaly compare the effect of Ostene® and gelfoam on bone healing. To our knowledge, the data in this report is one of few reports comparing the two materials, so more research is still needed. Our results show that Ostene® has an obvious effect on bone healing, and if it does not accelerate the healing process, it does not delay it either. We believe that this is because Ostene® does not aid the development of infection, which agrees with Wellisz *et al.,*[9] who reported that the bone healing process was not affected by the presence of Ostene® and it did not induce infection.[23] Regarding inflammatory infiltrations during the first 24 hr of healing, there was no significant difference between the three groups.

This further indicates that Ostene® did not impair the healing process, in agreement with the study of Vestergaard *et al.,*[22] in which they explained this effect by a reduction in cellular response to Ostene®, probably due to the quick elimination time. At 3 days post-intervention, there were significant differences between Ostene® and both gelfoam and control groups in the induction of inflammation. The number of inflammatory cells at 3 days post-intervention in the Ostene® group was higher and more specific with a high capacity for secreting more chemotactic factors, the process during the first 3 days appeared faster, and healing appear to take a shorter time in comparison with the other groups.

As Ostene® is absorbed within 48 hr,[24] at 7 days post-intervention it had disappeared and its effect had thus subsided, nonetheless the healing process and inflammatory response appeared better in Ostene® group as compared to gelfoam at 14 days of bone healing, as gelfoam may act as a mesh for entrapping bacteria; this may lead to abscess formation.[25,26] Regarding granulation tissue formation, statistically our result showed there was no significant differences between Ostene® and the other groups (gelfoam and control) along all the periods of healing. This may be because Ostene® has no direct effect on fibroblast cells or that the amount of Ostene® applied into the defect was not sufficient to produce an obvious effect on the establishment of granulation tissue, or that Ostene® dissolved too quickly. According to a study of Claes *et al.,*[27] in larger defect sizes (≥6mm) a large amount of connective tissue formation is seen.

Because the size of the defect in our study was small (5mm), this may explain why the granulation tissue was not seen clearly. A study by Gurcan *et al.,*[28] showed Ostene® had antifibrosis effect. This finding is in agreement with our results. Despite we showed no effect of Ostene® in granulation tissue formation, its effect on the initiation of osteoblast, osteoclast proliferation and new blood vessels formation was clear in histological examination.

Hence, Ostene® has an effect when compared with the other groups (gelfoam, control) considering osteoblast and osteoclast cells formation at 3 days post-intervention, as illustrated by the significant difference observed in formation of these types of cells. These findings agree with several studies in which Ostene® was found to be as absorbable as bone wax but without adverse effects on bone healing or osteogenesis.[6,29-31]

CONCLUSION.

Ostene® bone hemostatic agent is an effective agent that does not inhibit bone healing and enhances osteogenesis by initiating proliferation of osteoblast and osteoclast cells.

ISSN Online 0719-2479 - www.joralres.com © 2018

REFERENCES.

1. Schmidt-Bleek K, Petersen A, Dienelt A, Schwarz C, Duda GN. Initiation and early control of tissue regeneration - bone healing as a model system for tissue regeneration. Expert Opin Biol Ther. 2014;14(2):247–59.
2. Pivonka P, Dunstan CR. Role of mathematical modeling in bone fracture healing. Bonekey Rep. 2012;1:221.
3. Einhorn TA, Gerstenfeld LC. Fracture healing: mechanisms and interventions. Nat Rev Rheumatol. 2015;11(1):45–54.
4. Claes L, Recknagel S, Ignatius A. Fracture healing under healthy and inflammatory conditions. Nat Rev Rheumatol. 2012;8(3):133–43.
5. Spotnitz WD. Getting to hemostasis: general and thoracic surgical challenges. Tex Heart Inst J. 2012;39(6):868–70.
6. Doonquah L, Mitchell AD. Oral surgery for patients on anticoagulant therapy: current thoughts on patient management. Dent Clin North Am. 2012;56(1):25–41.
7. Armstrong JK, Han B, Kuwahara K, Yang Z, Magyar CE, Dry SM, Atti E, Tetradis S, Fisher TC. The effect of three hemostatic agents on early bone healing in an animal model. BMC Surg. 2010;10:37.
8. Wang MY, Armstrong JK, Fisher TC, Meiselman HJ, McComb GJ, Levy ML. A new, pluronic-based, bone hemostatic agent that does not impair osteogenesis. Neurosurgery. 2001;49(4):962–8.
9. Wellisz T, Armstrong JK, Cambridge J, An YH, Wen X, Kang Q, Hill CM, Fisher TC. The effects of a soluble polymer and bone wax on sternal healing in an animal model. Ann Thorac Surg. 2008;85(5):1776–80.
10. Achneck HE, Sileshi B, Jamiolkowski RM, Albala DM, Shapiro ML, Lawson JH. A comprehensive review of topical hemostatic agents: efficacy and recommendations for use. Ann Surg. 2010;251(2):217–28.
11. Kumar S. Local hemostatic agents in the management of bleeding in oral surgery. Asian J Pharm Clin Res. 2016;9(3):35–41.
12. Wellisz T, Armstrong JK, Cambridge J, Fisher TC. Ostene, a new water-soluble bone hemostasis agent. J Craniofac Surg. 2006;17(3):420–5.
13. Wong P, Johnson KJ, Warner RL, Merz SI, Kruger GH, Weitzel WF. Performance of Biopsy Needle With Therapeutic Injection System to Prevent Bleeding Complications. J Med Device. 2013;7(1):110021–7.
14. Palm MD, Altman JS. Topical hemostatic agents: a review. Dermatol Surg. 2008;34(4):431–45.
15. Spotnitz WD, Burks S. Hemostats, sealants, and adhesives: components of the surgical toolbox. Transfusion. 2008;48(7):1502–16.
16. Vyas KS, Saha SP. Comparison of hemostatic agents used in vascular surgery. Expert Opin Biol Ther. 2013;13(12):1663–72.
17. Hong YM, Loughlin KR. The use of hemostatic agents and sealants in urology. J Urol. 2006;176(6 Pt 1):2367–74.
18. Baek I, Lee YK, Park SJ, Bai CZ, Park JS, Kim DJ. Paclitaxel Coating Inhibits Inflammation Surrounding Subcutaneously Implanted Expanded Polytetrafluoroethylene (ePTFE) Hemodialysis Grafts in Rabbit Mode. Bull Korean Chem Soc. 2010;31(2):281–5.
19. Henke J, Astner S, Brill T, Eissner B, Busch R, Erhardt W. Comparative study of three intramuscular anaesthetic combinations (medetomidine/ketamine, medetomidine/fentanyl/midazolam and xylazine/ketamine) in rabbits. Vet Anaesth Analg. 2005;32(5):261–70.
20. Zheng LW, Wong MCM, Rabie ABM, Cheung LK. Evaluation of recombinant human bone morphogenetic protein-2 in mandibular distraction osteogenesis in rabbits: Effect of dosage and number of doses on formation of bone. Br J Oral Maxillofac Surg. 2006;44(6):487–94.
21. Lucaciu O, Gheban D, Soriţau O, Băciuţ M, Câmpian RS, Băciuţ G. Comparative assessment of bone regeneration by histometry and a histological scoring system. Rev Romana Med Lab. 2015;23(1):31–45.
22. Vestergaard RF, Bruel A, Thomsen JS, Hauge EM, Søballe K, Hasenkam JM. The Influence of Hemostatic Agents on Bone Healing After Sternotomy in a Porcine Model. Ann Thorac Surg. 2015;99:1005–12.
23. Wellisz T, An YH, Wen X, Kang Q, Hill CM, Armstrong JK. Infection rates and healing using bone wax and a soluble polymer material. Clin Orthop Relat Res. 2008;466(2):481–6.
24. Grindel JM, Jaworski T, Emanuele RM, Culbreth O. Pharmacokinetics of a novel surface-active agent, purified poloxamer 188, in rat, rabbit, dog and man. Biopharm Drug Dispos. 2002;23(3):87–103.
25. Schreiber MA, Neveleff DJ. Achieving hemostasis with topical hemostats: making clinically and economically appropriate decisions in the surgical and trauma settings. AORN J. 2011;94(5):S1–20.
26. Phippen M, Ulmer B, Wells M. Competency for Safe Patient Care During Operative and Invasive Procedures. 1st Ed. Denver, CO. USA: Competency & Credentialing Institute; 2009.
27. Claes LE, Heigele CA, Neidlinger-Wilke C, Kaspar D, Seidl W, Margevicius KJ, Augat P. Effects of mechanical factors on the fracture healing process. Clin Orthop Relat Res. 1998;(355 Suppl):S132–47.
28. Gurcan O, Gurcay AG, Kazanci A, Onder E, Senturk S, Bavbek M. Is the use of hemostatic matrix (Floseal) and alkylene oxide copolymer (Ostene) safe in spinal laminectomies? Peridural fibrosis assessment. Acta Orthop Traumatol Turc. 2017;51(2):165–8.
29. Das JM. Bone Wax in Neurosurgery: A Review. World Neurosurg. 2018;116:72–6.
30. Achneck HE, Sileshi B, Jamiolkowski RM, Albala DM, Shapiro ML, Lawson JH. A comprehensive review of topical hemostatic agents: efficacy and recommendations for use. Ann Surg. 2010;251(2):217–28.
31. Vestergaard RF, Jensen H, Vind-Kezunovic S, Jakobsen T, Søballe K, Hasenkam JM. Bone healing after median sternotomy: a comparison of two hemostatic devices. J Cardiothorac Surg. 2010;5:117.