

Experimental Study to Evaluate the Effect of Topical Hemostatic Agents on Healing of Bone Defects after Endodontic Microsurgical Procedures

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Abstract Hemostatics are agents used in surgery to control the bleeding. To make the right choice is a challenge to the dentist because he should weigh the benefits of these agents against its effect on bone healing. These agents act by different mechanism of action which effect wound healing. It is important to compare these mechanisms to make the right choice when we select these agents during surgery. **Objectives:** To evaluate the effects of topical hemostatic agents on healing of bone after endodontic microsurgical procedures using experimental animals model. **Materials and method:** The study was involving 300 patients (160) male, (140) female (10-45y) come to the Department of maxillofacial surgery/College of dentistry/University of Anbar (2000-2018) endodontic microsurgery. Local anesthesia was used (2% Lidocaine with 1:80.000 adrenalines). Alustat, Collagen sponge and adrenalin were tested for their hemostatic effect and tissue reactions, and were compared with untreated control. 60 New Zealand white rabbits were involved in the study, used for examination the tissue reaction to these agents. The animal were anesthetized by i.p ketamine 10mg with XYL-M2 xylazine 20mg. The bone defects were made using standardized aseptic technique. Three sided flap were made in the anterior mandible of the rabbits using scalpel. The bone defects (5 mm) were made using straight surgical hand piece and burs with constant irrigation by normal saline. The effect of the above agents (bleeding time and tissue reaction) was examined visually then compared with control. The biopsy were taken after 3 and 4 weeks then sent for histopathological examination. **Results:** Statistical analysis in [Table 1](#) & [Figure 5](#) indicate that the highest healing % was in collagen group (81.000%). While the lowest healing % was in Alustat group (72.2500%). The healing % for Adrenalin was (75.000%). The mean difference in healing % is significant at 0.01 & 0.05 level. **Conclusion:** Hemostasis in experimental bone defects is most effectively accomplished by using collagen. Understanding such mechanism is important for successful results.

Keywords: collagen, adrenalin, alustat, bone healing, hemostasis, endodontic surgery, topical hemostatics, bone necrosis, resorbable hemostatic agents, osteoid

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1. Introduction

The control of bleeding is important in surgery. In apical surgery it makes visualization of root end easy. It also facilitates application of root end filling materials. These agents should be used then it should be either removed or allowed to be resorbed with no effect on wound healing [1]. The surgeon should weigh the side effects and the benefits when using these materials based on mechanism of action and wound healing effects in endodontic microsurgery [2,3]. Resorbable hemostatic agent used recently because these agents not interfering with wound healing as shown in histological studies. [4] The biological stability of such agents is important for healing of periapical bone, surgical flap, blood vessels and nerves. Because these agents may leave unwanted tissues reactions if it used incorrectly. [5] Studies by Von et al on

the hemostatic effect of bone wax, ferric sulphate, and aluminum chloride. The additive effect of adding stasis to these agents has been more effective to stop bleeding. Because the use of such agent associated with inflammation and foreign body reaction. The use of such agents i.e, Expasyl TM is associated with freshening of surgical bone cavity with surgical bur after application and setting of root end filling material [6]. Collagen hemostatic sponge is made from lyophilized hydrolyzed collagen. It takes 15 days for complete resorbition with excellent bone healing effect. In surgery it uses for its hemostatic and wound healing ability, it may or may not remove after few days [7]. Gelatin is used as hemostatic agents in tooth extraction sockets and bone graft donor site, but it is associated with delay inflammatory reaction [9]. Adrenalin 1% could be added to gelatin to provide an additional hemostatic effect. Gelatin also promote aggregation of platelets and stop bleeding indirectly, but thrombin have direct action on the last sequence of clot formation. [10] Among many advantages

of hemostatics, it arrest bleeding, eliminate use of coagulants, decrease time of surgery, speed patients recovery. But it has side effects including foreign body reaction, chronic inflammation, infection and delay healing and granuloma formation. [11] Other modalities for control soft tissue and bone bleeding surgery are electrocautary. It act by coagulation and vesicular clumping with no foreign body reaction[12] Adrenalin is used widely in dentistry. It is a potent vasoconstrictor in oral cavity. It is sympathomimetic agent with α and β receptor action with rare local tissue reaction. Side effects of this drug include tissue ischemia and necrosis of bone, gingiva and sensory nerve. Which is dose related (0.1 and 0.01%). [13] In addition to cardiovascular complication of adrenalin due to β receptor action i.e., it increase cardiac output and increase pulse rate. This effect could be increase in endodontic microsurgery when adrenalin enter the circulation (1:100.000-1:80.000). The danger of intraosseous injection of adrenalin is more than mucosal routes. [14] The liquid form hemostatic agents used in endodontic microsurgery (i.e adrenalin, aluminum chloride liquid, ferric sulfate solution) require carrier agent (resorbable collagen sponge or cotton pellets) to take the excess of liquid out of surgery then to arrest bleeding and provide good healing. [15] Resorbable collagen sponge is mostly take in consideration because of space maintaining and biodegradable agents make this material to use in revascularization and repair of perforation in surgery. It provides good healing and plenty of fluid in surgical area and resorbed with no remaining material. [16] Electrocauterization or thermal cautery is efficient method to control bleeding in general surgery i.e, heat electrode passed through tissue to control bleeding or produce tissue destruction. It could be used in minor surgery or general surgery, plastic surgery, ect. [17] It action by using heat to induce coagulation and clumping of vesicle. It used to arrest bleeding in soft tissue and oozed bone surfaces, but resulted in thermal damage to the tissue. [18,19].

2. Materials and Method

2.1. Sample

The study was involve 300 patients (160) male, (140) female (10-45y) come to the Department of oral maxillofacial in our college between (June /2000-2018) for endodontic microsurgery.

2.2. Materials

Collagen sponge (Gelatamp) size (14×7×7mm) contains: Hardened gelatin Ph.Eur.9.5mg. Colloid silver Ph.Eur. 0.5mg, Alustat (25% aluminum chloride liquid), adrenalin (2% Lidocaine with 1:80.000 adrenalin) in dental Local anesthesia.

2.3. Method

Local anesthesia was used (2%Lidocaine with 1:80.000 adrenaline) for these patients. The surgical procedure was done and the following hemostatic modalities were tested for their hemostatic effect and tissue reactions, and were compared with untreated control: Alustat (25% Almmiun

chloride liquid), collagen sponge and adrenalin. These agents were tested visually (bleeding time and tissue reaction) then compared with control. 20 New Zealand White rabbits were used for histopathological examination. The rabbits were anesthetized by i.p ketamine 10mg with XYL-M2 xylazine 20mg. The bone defects were made using standardized aseptic technique. Three sided flap were made in the anterior mandible of the rabbits using scalpel. The bone defects (5 mm) were made using straight surgical hand piece and burs with constant irrigation by normal saline. The above agents were tested visually (bleeding and tissue reaction) and were compared with control. The biopsy were taken after 2 and 4 weeks after healing in 10% formalin then sent for histopathological examination.

3. Results

1-Clinical examination: Clinical examination of the treated area show good hemostatic effect among all the treated groups, the hemostatic effect is most effective and favorable among the group treated with collagen in comparison with other groups.

2-Histopathological examination: Histopathological examination of the biopsy specimen show osteoid formation in collagen treated group after 2 weeks. While bone degeneration and necrosis in group treated by Alustat. The study indicate that there is foreign body reaction (tissue granuloma) and necrosis in the animals treated by topical aluminum chloride liquid after 2 weeks.



Figure 1. The biopsy specimen after 2 weeks

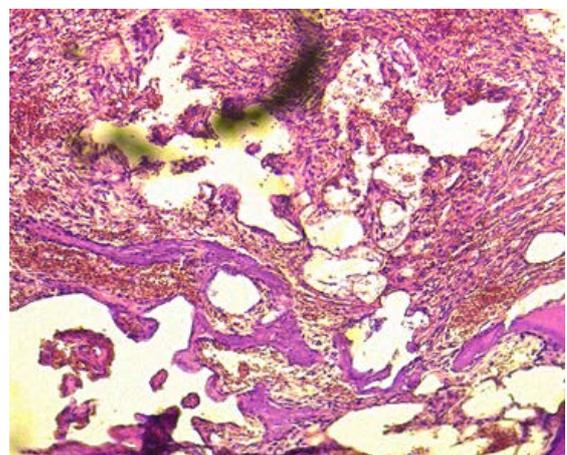


Figure 2. The blood clot in the control defect

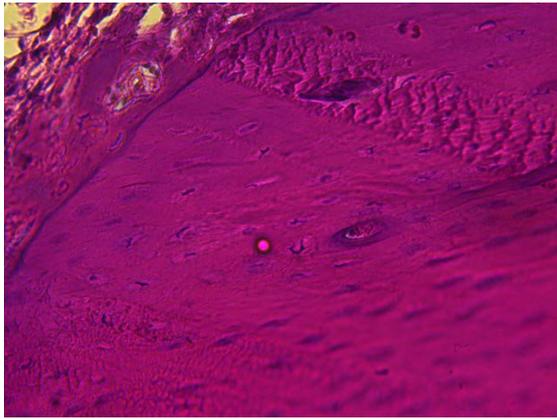


Figure 3. Show the osteoid formation induced by collagen after 2 weeks

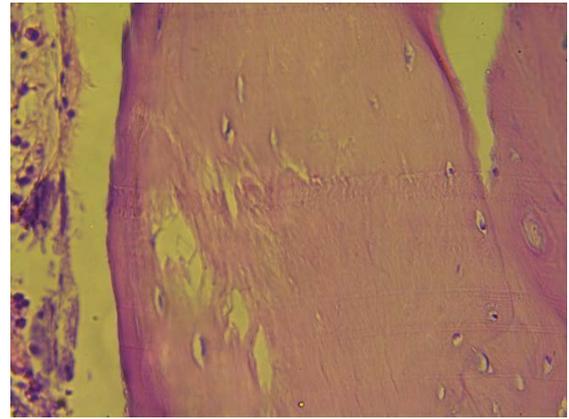


Figure 4. Show the bone necrosis induced by Alustat after 2 weeks

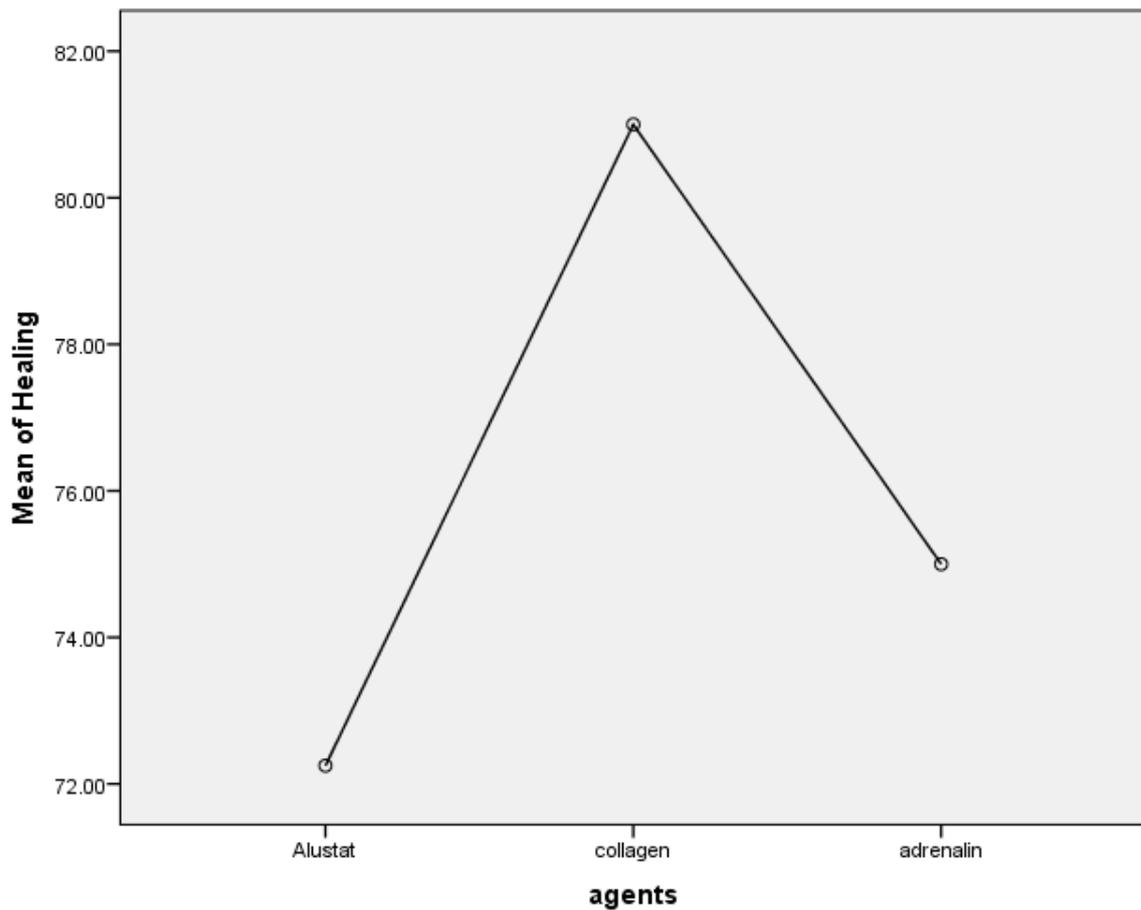


Figure 5. The healing % of different hemostatic agents

Table 1. Show healing % of different hemostatic agents

Hemostatic agents	Healing perc.
Alustat	72.2500%
collagen sponge	81.0000%
adrenalin	75.0000%
Total	76.0833%

3-Statistical Results

The results of statistical analysis in Table 1, Table 2 & Figure 5 show that the higher % of healing was in collagen treated group (81%)when compared with other groups (75%) & (72%) in adrenalin and Alustat

respectively. There is significant difference in mean healing % at 0.05 &0.01 levels. Table 3 show multiple comparison with the group with mean difference is significant at 0.05 level.

Table 2. The Anova -test of analysis of variance in mean healing % of different agents

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	160.167	2	80.083	17.687	.001
Within Groups	40.750	9	4.528		
Total	200.917	11			

*The mean difference is significant at 0.05 and 0.01 level.

Table 3. The multiple comparison in mean of healing % of different agents

Dependent Variable: VAR00001						
LSD						
[I] VAR00002	[J] VAR00002	Mean Difference [I-J]	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-8.75000*	1.50462	.000	-12.1537-	-5.3463-
	3.00	-2.75000-	1.50462	.101	-6.1537-	.6537
2.00	1.00	8.75000*	1.50462	.000	5.3463	12.1537
	3.00	6.00000*	1.50462	.003	2.5963	9.4037
3.00	1.00	2.75000	1.50462	.101	-.6537-	6.1537
	2.00	-6.00000*	1.50462	.003	-9.4037-	-2.5963-

*. The mean difference is significant at the 0.05 level.

This study was done to evaluate clinically the hemostatic efficiency and tissue reactions of topical hemostatic agents used in endodontic microsurgery. Because right use and choice of hemostatic agent is mandatory in our surgery for good prognosis. These agents have different characteristics and mechanism of action which affect wound healing. [20] The statistical analysis in Table 1 and Figure 5 indicate that the highest healing % was in collagen group (81.0000%). Because of the favorable effect induced by collagen during surgery on the bone and soft tissue. Its osteoid forming capacity and soft tissue healing make it used widely in surgery as hemostatic agents in comparison with Alustat and Adrenalin. While the lowest healing % was in Alustat (72,2500%) in comparison with other groups because of the unfavorable tissue reaction induced by Alustat. It cause tissue necrosis and local foreign body reaction [granuloma] after 2 weeks as proved in experimental animal. So during surgery before suturing of flap it is necessary to remove superficial layer of bone by bone bur. While the healing % of adrenalin was (75.0000%) because although adrenalin is widely used in dentistry as vasoconstrictor in dental local anesthesia it act on α receptor on blood vessels and cause vasoconstriction but it lead to tissue necrosis and ischemia for bone and gingiva. Table 2 show the mean difference in healing percent for different agents is significant at 0.05 & 0.01 level. Statistical results in Table 3 show the multiple comparison in mean value of healing % with mean difference is significant difference at 0.05 level. [21] Collagen is used widely in surgery to control bleeding after tooth extraction. It also placed on donor site after bone graft harvesting. It cause delay inflammatory response in area treated with collagen in comparison to untreated control defects. Collagen is totally resorbed by macrophages after period of time as proved in animal's mandible. [22] Adrenalin could be added to collagen to increase the hemostatic effect and to allow placement and setting of dentin bonded root end fillings. The results of our study indicate that the addition of adrenalin to collagen have limited increase on its hemostatic effect. [23] Adrenalin is a sympathomimetic agents, it cause vasoconstriction but when used as hemostatic agents it cause tissue ischemia and necrosis. This is agreed to other studies which proved that there no significant differences. While the addition of adrenalin to collagen give some benefit without reaching the other groups. [24] Studies on using of adrenalin in low

concentration (1:100.000-1:80.000) injection in ear, nose and hand and fingers is safe. While studies by Denkler who evaluate 21 patients have digital gangrene followed adrenalin injection and sensory nerve damage. Review literature 2000 suggest that uncontrolled dilution of adrenalin as one of reasons. [25] Alustat is a topical aluminum chloride. It used nowadays in liquid form to arrest bleeding during endodontic microsurgery, but were associated with unfavorable tissue response ie., presence of bone necrosis, inflammation and the absence of bone repair that not recover with the time. Its action is similar to electrocautery. [26] We have to consider degree of proliferation of granulation tissue and tissue necrosis and inflammation when compare the tissue healing action of hemostatic agent in surgery. [27] The addition of hemostatic agents to Alustat will increase it hemostatic effect. Evidence on tissue reactions to aluminum chloride paste is limited, while studies on using topical application of liquid form aluminum chloride reveals stated inflammatory response [28]. The results of many studies indicate that these reactions could be significantly reduced by freshening of the bone cavity using small surgical round bur and to avoid touch and direct water irrigation for the cut root surface treated with MTA root end filling material. Cauterization other efficient means to control bleeding in surgery similar to the effect of combination of Alustat 25% with hemostatic. However, studies indicate that it may cause delay healing of bone and limited bone formation after 12 weeks in compared with control defect and defect treated with Alustat 25% and hemostatic. [29] Before suturing of flap we should to remove the coagulated tissue by curette. And to remove the superficial layer of bone treated with cauterization by bur in the same manner to Alustat 25% & hemostatic treated defects. [30] It is of importance to mention that absorbable collagen sponge and cotton pellet used as carrier for topical hemostatic agent. [31]

4. Conclusion

Control of bleeding is important in surgery. Understanding the mechanism of action of topical hemostatic agents is important. Because of unfavorable tissue response by some agents. Selection of best agent is important to achieve good outcome.

5. Recommendations

Selection of best hemostatic agents is important in surgery to avoid the unwanted effect on the vital bone and soft tissue structures.

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