(International Print/Online Journal)

SJIF IMPACT FACTOR: 5.565
PUBMED-National Library of Medicine ID-101739732

ISSN (Print): 2209-2870
ISSN (Online): 2209-2862

International Journal of Medical Science and Current Research (IJMSCR)
Available online at: www.ijmscr.com
Volume 5, Issue 3, Page No: 773-781
May-June 2022

IJMSCR

Comparative Evaluation Of Fluid Absorbency Of Botroclot With Different Gingival Retraction Medicaments – An In Vitro Study

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Type of Publication: Original Research Paper
Conflicts of Interest: Nil

Abstract

Purpose*:* This study aims to know the effect of various medicaments and Botroclot on fluid absorbency of retraction cords and know whether the retraction cord's thickness influences its fluid absorbency.

Materials and Methods: Knitted gingival retraction cords (SURE-Cord) of two different thicknesses (No.0 and No.1) were used. The medicaments used were 25% $AlCl_3$ & 20% $FeCl_3$, Adrenaline bitartrate, and Botroclot. The absorbency was tested using two fluids: artificial saliva and human plasma. The cords were soaked for 20 minutes to measure the optimum soaking time, and the weights were measured. Later, they were immersed in artificial saliva and human plasma for 10 minutes, and their weights were measured again. The amount of fluid absorbed was determined by subtracting the weight before immersion from the weight after immersion.

Results: The results implied that 20 minutes of soaking time of cords in Botroclot was optimal for its saturation before use. There was no correlation between the cord's thickness and its saturation. There was no significant difference between the fluid absorbency of cords immersed in botroclot and those immersed in other retraction medicaments.

Conclusion: Within the study's limitations, it can be concluded that Botroclot can be used as a viable gingival retraction medicament.

Keywords: Gingival retraction, Knitted gingival retraction cords, 25% $AlCl_3$, 20% $FeCl_3$, Adrenaline bitartrate, and Botroclot

Introduction:

Margin integrity is one of the critical criteria of the principles of tooth preparation. One should maintain a dry field to make an accurate and precise impression of the finish line configuration in fixed prosthetic treatments. Adequate fluid control in the gingival sulcus is needed to make an exact impression. Several techniques and methods have been used for displacing the gingival tissues. They include mechanical, mechano-chemical methods, rotary curettage, and electrosurgery. There are two parts in managing gingival tissues: the first is deflecting the marginal gingiva away from the prepared tooth and controlling the moisture in the gingival sulcus. The gingival tissues are strayed away from the tooth using gingival retraction cords, creating lateral and vertical space for sufficient bulk of the impression material. Managing sulcular fluid, salivary contamination, and gingival bleeding is absolutely crucial when hydrophobic impressions like polyvinyl siloxane are used. The most commonly used method is the mechanochemical method, where the gingival retraction cords are immersed in various medicaments. The retraction cords physically

displace the marginal gingiva and keep the chemicals in close contact with the tissues.[1] Chemical retraction agents have been used for this purpose and are largely divided into vasoconstrictors and astringents. Vasoconstrictors includes alpha and beta-adrenergic (0.1/1.0/8.0% epinephrine) and alpha-adrenergic (0.05% tetra hydrazine HCl, 0.05% oxymetazoline HCl, 0.025% phenyl epinephrine HCl). Astringents include chlorides of aluminium (10, 20, 25% $AlCl_3$) and iron (8-20% $FeCl_3$) and sulphates of aluminium [20-25% $Al_2(SO_4)_3$] and iron (15.5, 20, and 12.7 $Fe(SO)_4$). All the currently known astringents cause some local temporary tissue damage, and vasoconstrictors elicit negative systemic effects. Botroclot is a nontoxic hemocogulant fraction of snake venom. It is known for its use in the arrest of bleeding in cases of extraction sockets. The purpose of this study is to compare and evaluate the soaking time of various medicaments and Botroclot, to know the effect of these medicaments and Botroclot on fluid absorbency of retraction cords, and to know whether the thickness of the retraction cord influences its fluid absorbency.

Materials And Methodology:

This study was conducted using four commercially available retraction medicaments. Astringent used was 25%$AlCl_3$ (Prevest Denpro Hemostal gel), and styptic used was 20% $FeCl_3$ (DentRetract, Prime Dental Products Pvt. Ltd). Vasoconstrictor used was veils of Adrenaline bitartrate, and hemocogulase used was Botroclot (Juggat Pharma). Non-impregnated knitted retraction cords (Sure-Cord) of sizes 0 and 1 were used. The absorbency was tested using two fluids: artificial saliva (Wet Mouth) and human plasma. An electronic weighing balance was used to measure the weights. The total samples taken were 200 and were divided into the different groups: (Figure 1)

Optimal Soaking time measurement: For this, a 5cm length of each thickness retraction cord was cut and saturated with all four retraction medicaments. Before immersion, the cords were manually pulled against a piece of clean filter paper between the thumb and index finger. The retraction cord's weight was recorded at 20 minutes time intervals.

Fluid Absorbency: After soaking the retraction cords (thickness 0 and 1) with the retraction medicaments for 20 minutes, they are immersed in artificial saliva and human plasma for 10 minutes. Their weights were measured again. The amount of fluid absorbed was determined by subtracting the weight before immersion from the weight after immersion. Dry, untreated cords were taken as controls, and they were immersed similarly in artificial saliva, and human plasma and weights were recorded before and after immersion.

Results:

The weights of the retraction cord were measured at 20 minutes time intervals after soaking them in the four chemicals. The mean and standard deviation of the weight in grams were presented (Table 1).

The results showed that the cord (No.0) soaked in botroclot showed the highest weight (0.0197), followed by epinephrine (0.0195), 25% $AlCl_3$ (0.0188), and 20% $FeCl_3$ (0.0151). Similarly, the cord (No.1) soaked in botroclot showed the highest weight (0.0327), followed by epinephrine (0.0314), 25% $AlCl_3$ (0.0294), and 20% $FeCl_3$ (0.0286). One-way ANOVA was done to compare the soaking time of each medicament. The results showed no statistical significance among the weights of retraction cords after soaking them in the four retraction medicaments (Table 1)

The amount of artificial saliva absorbed by the cords increased from controls (0.0039, 0.0067), to 20% $FeCl_3$ (0.0103, 0.0127), 25% $AlCl_3$ (0.0128, 0.0148), epinephrine (0.0135, 0.0178) and botroclot (0.0141, 0.0178). The amount of human plasma absorbed increased from controls (0.0042, 0.0076), to 20% $FeCl_3$ (0.0109, 0.0146), 25% $AlCl_3$ (0.0117, 0.0155), botroclot (0.0133, 0.0172) and epinephrine (0.0143, 0.0178).

One-way ANOVA comparison showed the amount of fluid absorbed by dry retraction cords and the cords impregnated with medicaments was statistically significant (Table 2). The amount of fluid absorbed increased with the increase in the thickness of the cord. However, this increase was not statistically significant (p-value > 0.05) when tested with the Spearman correlation ratio (Table 2).

Post-hoc Bonferroni test was done to analyze pair-wise comparisons (Table 3). The results showed a significant difference (p-value < 0.05) of fluid

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absorbed among dry, untreated cords and the cords immersed in botroclot, 25% $AlCl_3$, 20% $FeCl_3$, and epinephrine. However, no such statistical significance was seen when the four chemicals (botroclot, 25% $AlCl_3$, 20% $FeCl_3$, and epinephrine) were compared (Table 3).

Discussion:

For optimal gingival retraction, soaking time in the medicament is essential for the ingress of the medicament into the cord. Hemostatic action depends on the amount of medicament absorbed by the cord, further dependent on the cord's thickness, length, and structure.[2] The study conducted by Csempesz et al. reported that 20 minutes of soaking time was necessary for saturation of the cords with the medicaments prior to the use.[3] Before immersing the cords in the medicaments, they were manually pressed to remove any air inclusions from the inner pores and surface of the cords. Previous studies reported that the omission of this step substantially hindered the moistening of the cord.

In the present study, soaking time was analyzed by measuring the weights of the cord after immersion in the medicaments. The highest weight for both thickness cords was seen when immersed in botroclot and epinephrine than in 25% $AlCl_3$ or 20% $FeCl_3$. The reason for this could be the relatively thinner consistency and viscosity of epinephrine and botroclot when compared to astringents like 25% $AlCl_3$ or 20% $FeCl_3$. The results indicated no significant difference in the weights of retraction cords after 20 minutes of immersion in the medicaments. This outcome implies that, like other medicaments, 20 minutes of soaking the cords in botroclot is optimal for its saturation before its use. In the present study, the saturation levels of the solutions did not show a significant correlation with cord thickness. This finding was consistent with the study conducted by Csempesz et al.[3]

Studies were done earlier to determine whether the medicaments hamper or enhance the fluid absorbency of retraction cords. Runyan DA et al. investigated and determined that soaking retraction cord in aluminium chloride solution before placing it into gingival sulci does increase the cord's ability to absorb fluid.[4] Vishnubhotla et al. evaluated the effect of 10% aluminium chloride and 15.5% ferric sulfate on fluid absorbency of the retraction cord.[5] They concluded that 15.5% ferric sulfate was a better medicament for the absorption of fluid. Kansal et al. evaluated the effect of potash alum and 21% ferric sulfate on kinetic absorbency of retraction cord. They reported that kinetic absorbency increased in impregnated cords than dry retraction cords. They also concluded that potash alum showed the most favorable results.[6]

In the present study, artificial saliva and human plasma were chosen to simulate saliva and crevicular fluid in vitro. Human plasma contains proteins similar to gingival crevicular fluid and blood.[4] The fluid absorbency was statistically higher in retraction cords soaked in medicaments than dry, non-impregnated cords. This finding was consistent with the previous studies.[4–6] However, there was no statistical significance in fluid absorbency among the retraction cords immersed in the four medicaments. This result implies that botroclot is equally effective in absorbing fluids like other gingival retraction medicaments.

The rationale behind proposing a new gingival retraction medicament is that the currently known chemicals have some adverse side effects. Most of the chemicals are used to restrict gingival hemorrhage. Amongst these chemicals, epinephrine in various concentrations (2%, 4%, and 8% racemic epinephrine) is most commonly used. There is still some conflict among practitioners and researchers regarding the systemic effects of epinephrine retraction systems.[7–8] Brill reported that the sulcular epithelium is a semi-permeable membrane. It allows the passage of different-sized molecules, depending on the state of equilibrium. It was stated that epinephrine could enter the vascular bed by osmosis and subsequently affect the various organ systems in the human body.[9] Undesired systemic effects may be encountered in cases of the cumulative impact of epinephrine from sources other than the retraction cords. These sources might be from epinephrine administered via the local anesthetic solution and endogenously produced epinephrine due to stress during the dental procedure.[10–14] These above-mentioned undesired effects can occur in any patient, but most likely in patients with predisposing diseases like cardiovascular disease, hyperthyroidism, diabetes, and hypersensitivity to epinephrine. The

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dentist has to substitute another retraction agent in patients taking mono-amine or tricyclic antidepressants, rauwolfia compounds, ganglionic blockers, cocaine, or other epinephrine-potentiating drugs.[16–17]

Astringents are the next big group of gingival retraction systems. Astringent is defined as "a drug that causes cells to shrink by precipitating proteins from their surfaces," according to Concise Medical Dictionary (CMD).[18] Astringents produce hemostasis by causing tissue contraction followed by blood coagulation in the vessels in the local area. Styptics may be considered as a concentrated form of an astringent.[19] Aluminium chloride ($AlCl_3$) is by far the most commonly used astringents. It is used in concentrations of 5 - 25%. The acidic property of aluminum chloride causes a reaction with blood proteins, which creates a barrier by coagulated proteins and prevents the outflow of blood from vessels.[20] Ferric chloride is a styptic and is used in concentrations of 8 – 20%. In the present study, 25% $AlCl_3$ and 20% $FeCl_3$ were used. Both of these retraction chemicals are highly acidic. Local, temporary gingival tissue damage, irritation, inflammation, desquamation of sulcular epithelium, and post-operative sensitivity can occur due to high acidity. Buffering of concentrated forms reduces acid concentration, but it also decreases their hemostatic potential. Also, these agents seem to alter the dentinal surface properties by making them more resistant to acid etching.[21–22] Another significant adverse effect of ferric compounds, is the temporary staining of soft tissues to a bluish to brown/black color due to its iron content. Even though the gingiva returns to its normal pink appearance after 1 to 2 days, it might disturb the patient.[23–24]

Botroclot is a nontoxic systemic hemocoagulant fraction of venom obtained from the Brazilian snake Bothrops-jararaca or atrox. It has procoagulants that convert fibrinogen into fibrin. Hemocoagulase not only arrests capillary bleeding within one minute but also promotes wound healing. It establishes capillaries in wound space which encourages wound healing and enhances epithelization to reduce healing time. It markedly reduces inflammation, infection, and localized collection of blood at the site of injury.[25] Kiruthika et al. reported hemocoagulase to be equally effective to tranexamic acid in the arrest of bleeding in post-dental extraction sockets.[26] In the present study, there was no significant difference between the optimal soaking time and the amount of fluid absorbency by cords impregnated with botroclot and other retraction medicaments. So, Botroclot can be considered as a workable gingival retraction medicament. It is economical, available for topical use. It is not acidic, hence not requiring any buffering, and doesn't cause any local tissue irritation. It has no reports of systemic adverse effects. However, it is contraindicated in patients with a tendency for intravascular coagulation and venous and arterial thrombosis.[27–28]

Limitations:

1. As it is an in vitro study, the biologic environment of the oral mucosa could not be simulated.

2. Human plasma differs from GCF in a few high molecular weight proteins, and artificial saliva doesn't contain all the components in saliva.

3. Further investigation is required concerning the compatibility of the botroclot with impression materials.

Conclusion:

Within the study's limitations, it can be concluded that,

1. Botroclot, along with other medicaments, increased the fluid absorbency of the cords.

There was no increase in the saturation of the cords with the increase in cord thickness.

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Figure 1 : Schematic Representation of Distribution of Samples (n=200)

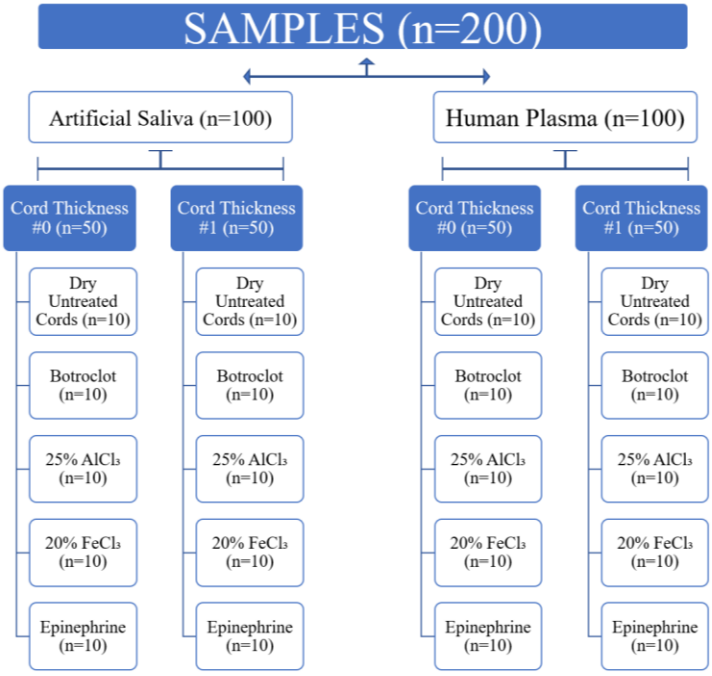

Figure 2 : Gingival retraction medicaments : 25%$AlCl_3$ (Prevest Denpro Hemostal gel), 20% $FeCl_3$ (*DentRetract*, Prime Dental Products Pvt. Ltd). Adrenaline bitartrate, and hemocogulase Botroclot (Juggat Pharma)

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Figure 3 : Human plasma, artificial saliva and retraction cord (Sure-Cord)

Table 1 : Comparison of Soaking time of cords of thickness #0 and #1 with different gingival retraction medicaments by One-way ANOVA analysis

Cord Thickness	One-way ANOVA				
	Groups	Mean	SD	f-ratio	p-value
CORD #0	Botroclot	0.0197	0.0052	1.6849	Insignificant
	25% $AlCl_3$	0.0188	0.0057		
	20% $FeCl_3$	0.0151	0.004		
	Epinephrine	0.0195	0.0059		
CORD #0	Botroclot	0.327	0.0079	0.9853	Insignificant
	25% $AlCl_3$	0.294	0.0056		
	20% $FeCl_3$	0.286	0.0038		
	Epinephrine	0.314	0.0064		

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Table 2 : Comparison of Fluid absorbency of the samples among different gingival medicaments by One-way ANOVA analysis

SAMPLE	Group	VISCOSITY (Pa-s)				RETENTIVE ABILITY (kPa)			
		Mean	SD	f-ratio value	p-value	Mean	SD	f-ratio value	p-value
FIXON POWDER	Control	2635	63.0507	73.92	0.00001	28.5	4.9318	6.3914	0.0004
	Cephalexin	2640.2	158.5313			28.1	5.0651		
	Nystatin	2642.4	122.1722			28.1	13701		
	Chlorhexidine	3271	34.053			35.5	3.3417		
	Silver Nanoparticles	2647.4	91.9108			28.2	4.2895		
SECURE POWDER	Control	2669.4	63.1844	145.91	0.00001	28.5	1.9003	8.444	0.000036
	Cephalexin	2663.4	30.4018			28.5	4.3525		
	Nystatin	2685.1	103.1476			28.1	4.5326		
	Chlorhexidine	3275.9	70.577			35.9	3.035		
	Silver Nanoparticles	2686.4	64.457			28.1	4.401		
FIXON CREAM	Control	4390.1	65.3613	0.0524	0.994	40.2	2.2509	0.393	0.812
	Cephalexin	4398.7	64.4447			39.3	2.2136		
	Nystatin	4390.3	100.8366			39.8	2.044		
	Chlorhexidine	4387.4	103.2098			39.3	2.2136		
	Silver Nanoparticles	4382	75.7574			39	3.1269		
SECURE CREAM	Control	4403.9	52.4541	0.0804	0.988	40.4	2.011	0.4382	0.7803
	Cephalexin	4401.4	101.6379			39.2	1.8738		
	Nystatin	4409.1	78.976			39.2	4.0497		
	Chlorhexidine	4390.3	100.8366			39.1	2.1318		
	Silver Nanoparticles	4406.7	60.314			39.2	2.4404		

Table 3 : Post-hoc Bonferroni Comparison of the Treatment Pairs in artificial saliva and in human saliva

Treatment Pairs	Bonferroni and Holm TT-statistics				Inference
	In Artificial Saliva		In Human Plasma		p-value
	CORD #0	CORD #1	CORD #0	CORD #1	
Control vs Botroclot	4.7172	5.7506	4.5837	5.433	** p<0.01
Control vs 25% $AlCl_3$	4.116	4.1964	3.7778	4.4709	** p<0.01
Control vs 20% $FeCl_3$	2.9598	3.1084	3.3748	3.9616	* p<0.05
Control vs Epinephrine	4.4397	5.8024	5.0874	5.7726	** p<0.01
Botroclot vs 25% $AlCl_3$	0.6012	1.5542	0.8059	0.9621	insignificant
Botroclot vs 20% $FeCl_3$	1.7574	2.6422	1.2089	1.4714	insignificant
Botroclot vs Epinephrine	0.2775	0.0518	0.5037	0.3396	insignificant
25% $AlCl_3$ vs 20% $FeCl_3$	1.1562	1.088	0.403	0.5093	insignificant
25% $AlCl_3$ vs Epinephrine	0.3237	1.606	1.3096	1.3017	insignificant
20% $FelCl_3$ vs Epinephrine	1.4799	2.694	1.7126	1.811	insignificant

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