An Assessment of the Effect of Chemical Impregnation on the Tensile Strength of Gingival Retraction Cords Associated with Scanning Electron Microscopic Evaluation

Sakshi Madhok[1],K.Kumaraswamy[2],Saksham Madhok [3]

ABSTRACT

Background & Objectives: Gingival retraction may damage the sulcular tissues irreversibly. Cord tearing during insertion or removal results in shreds being left behind within the gingival sulcus and supra-alveolar connective tissue. This has been attributed to the deficient tensile strength of the cords. It is crucial that retraction cords have satisfactory physico-chemical properties. There is much speculation in literature regarding the effect of hydration, types of cords, diameters of cords, effect of different chemical impregnating agents, variations in the concentrations of impregnating agents and the time for which cords are immersed in them, on the physico-chemical properties of retraction cords. The aim of this study is to assess the effect of chemical impregnation and its concentration variation on the tensile strength of cords & simultaneously evaluate its effect on the ultrastructure of cords using a scanning electron microscope (SEM).

Methods : 105 specimens of braided standard cotton cord each 10 centimeters in length were cut and randomly allocated to 7 groups (15 in each group). Specimens from Groups I to III and Groups IV to VI were impregnated with varying concentrations of Aluminium Sulfate (AS) and Ferric Sulfate (FS) respectively. Group VII was the control group. Each specimen was then subjected to tensile loading in an Instron machine and the readings when the specimens failed were recorded. Additional 2 specimens per group were taken for SEM Evaluation.

Results: The effect of impregnating agent and its concentration variation were analyzed by intra and inter group comparisons. Control group had maximum tensile strength & Group VI had minimal tensile strength. AS and FS impregnation led to a significant decrease in tensile strength of the specimens.

Conclusion: Within the limitations of this study, chemical impregnation negatively affects the ultrastructure of the retraction cords by reducing their structural integrity thereby hampering their physico- mechanical properties and weakening them.

Keywords: Retraction Agents, Scanning Electron Microscope, Tensile Strength,Gingival Retraction Cord.

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INTRODUCTION

Tooth Preparations for fixed prosthodontic restorations frequently include subgingival margins or finish line placement. Meticulous management of gingival soft tissue is a must to obtain satisfactory impression of the subgingivally placed finish lines[1,2]. Efficient & effective tissue displacement or gingival retraction allows sufficient impression material to be injected into the expanded gingival crevice to produce an acceptable impression[3]. This is important for the restoration to have a suitable emergence profile with well adapted gingival margins[4].

Various authors have proposed different methods of gingival retraction. These methods can be classified into simple mechanical [5,6,7] involving placement of a string into the gingival sulcus, chemo-mechanical[6,7,8] involving treatment of string with chemical impregnating agents prior to placement into the gingival sulcus, rotary gingival curettage or gingitage[9,10,11] involving the use of special diamond stones to remove the sulcular epithelium & electro-surgery[12,13] involving removal of a ribbon of gingival tissue from the sulcus around the cavity margin with dental electrodes. Amongst these four categories the chemo - mechanical method of gingival retraction is the most popular[14,15].

The various configurations of retraction cords available for chemo mechanical retractions are twisted, knitted & braided[16,17]. These are available in preimpregnated or non impregnated forms and the various chemical impregnating agents reported to be used include Caustic Chemicals (Sulphonic acid, Trichloroacetic acid, Negatol or Zinc Chloride)[2,18,19], Vasoconstrictors (8% Racemic Epinephrine)[14,20], Sympathomimetic Amines (0.05% Tetrahydrozoline hydrochloride, 0.05% Oxymetazoline hydrochloride or 0.25% Phenylephrine hydrochloride) [1] & Astringents 5 - 25% Aluminium chloride, 5 - 25% Aluminium sulfate, 100% Potassium Aluminium sulfate, 13.3% Ferric sulfate/subsulfate or 20-60%Tannic acid)[8,14,21].

However, this technique-sensitive method of gingival retraction if not carried out meticulously may result in injury to the gingivae and cause irreversible damage to the sulcular tissues[19]. It may cause degeneration of the underlying periodontium and delayed wound healing[3]. The effect of retraction cords on the gingival health is dependent on contact time of the cord with gingival sulcus and the chemical impregnating agent used for retraction[19,22]. Optimal contact time required to achieve a crevicular width of 0.2 mm with minimal gingival tissue damage is 4 minutes[23]. However this contact time is at times increased as the cords tear during insertion or more commonly during removal[24], resulting in shreds and frays of cotton fibers being embedded within the supra alveolar connective tissue apical to the cemento - enamel junction[25]. This tearing of retraction cords within the gingival sulcus has been investigated and is supposed to be correlated to their deficient tensile strength[24].

The need for retraction cords to have satisfactory physico-chemical properties has been emphasized[3]. But neither the effect of hydration, types, diameters or chemical nature of retraction cords nor the effect of

different chemical impregnating agents, variations in their concentrations and the time for which cords are immersed in them, on the physico-chemical properties of retraction cords have been evaluated adequately. Scanning electron microscopic studies have compared the ultrastructure of commercial non-impregnated cords to that of a synthetic biocompatible polymer[4], however the effect of chemical impregnating agents on the ultrastructure of cords has not been evaluated though it is the most commonly used method.

HENCE THE PURPOSE OF THE PRESENT STUDY IS

1. To evaluate the effect of varying concentrations of different impregnating agents on the tensile strength of gingival retraction cords.
2. To evaluate the effect of impregnating agents on the ultra structure of gingival retraction cords using scanning electron microscope

METHODOLOGY

In this study standard cotton cord specimens impregnated chemically with various concentrations of different chemical impregnating agents were used.

METHOD OF COLLECTION OF SAMPLE

105 specimens of braided standard cotton cord were cut from a bundle of cord of size 0 provided by the manufacturer. Each specimen was cut with scissors to a length of 10 centimeters as measured with a metallic measuring scale .

All the specimen were measured and cut to the determined length by a single operator. The specimens were randomly allocated to 7 different groups based on the type and the concentration of the chemical impregnating agent so as to have 15 specimens in each group. Specimens from Groups I to III were impregnated with varying concentrations of AS, while specimens from Groups IV to VI were impregnated with varying concentrations of FS. Group VII was the control group (Table I).

Table.1:Groups & their corresponding Impregnating Agents and Concentrations

Group	No. of Specimens	Impregnating Agent	Concentration (mg)
I	15	AS	200
II	15	AS	264
III	15	AS	400
IV	15	FS	105
V	15	FS	155
VI	15	FS	205
VII	15	-	-

PREPARATION OF IMPREGNATING SOLUTION

105 test tubes were taken i.e. 1 test tube for each specimen and arranged in test tube stands. Fresh double deionised water (DDW) was prepared in the deionization chamber by passing water twice through it and collected in a beaker. 1 ml of this DDW was dispensed into each of the test tubes using a millipipette . 105 test tubes containing DDW were randomly allocated to each of the 7 groups and labeled accordingly.

An electronic weighing balance was used to weigh appropriate amounts of the chemical impregnating agents i.e. AS and FS. A steel spatula was used to transfer small amounts of chemical impregnating agents on to a butter paper placed over the electronic weighing balance till the electronic scale showed the required weight. It was then transferred to the test tube of the respective group . Simultaneously 200 mg, 264 mg, 400 mg of AS & 105mg, 155 mg, 205 mg of FS, each were measured 15 times and subsequently transferred to the test tubes of their respective groups.

After the addition of the chemical impregnating agents to the test tubes, each test tube was centrifuged on a cyclomixer so as to achieve proper mixing of the chemical impregnating agents in DDW, as they were not easily miscible in DDW. Each test tube was kept on the cyclomixer until a saturated solution for impregnation was achieved.

PREPARATION OF THE SPECIMENS

Upon achieving complete dissolution of the chemical impregnating agent the specimens were immersed in the test tubes with a tweezers and covered with sterile cotton swabs to avoid contamination. Care was taken to ensure that every specimen was entirely dipped in the impregnating solution. The specimens were kept immersed in the impregnating solutions for 24 hours in a clean place.

105 small plastic containers were cleaned and sterilized by keeping them within the laminar flow cabinet (LFC) for 3 hours under ultraviolet rays . These plastic containers were labeled similar to the test tubes so as to aid in transfer of the specimens from the test tubes to these plastic containers. After 24 hours, each specimen was removed from the test tube and transferred to its corresponding plastic container using a tweezer . These plastic boxes containing specimens were kept for drying under clean and sterile condition in LFC for 24 hours. Temperature within the LFC was maintained at 23 Degree Celsius. Relative humidity was also maintained throughout this process.

Upon completion of 24 hours the specimens were removed from LFC. Non powdered latex gloves were used to handle the cords all throughout the procedure. 2 Specimens per group were taken in addition to above mentioned specimens for SEM evaluation. The control cords were also treated in a manner similar to that described above but without any chemical impregnating agent

TENSILE STRENGTH EVALUATION

Each dried specimen was then placed between the pneumatic grips and subjected to tensile loading in a universal testing machine calibrated to full load at 5 to 10 kg and cross head speed of 1 mm/min (Fig. 1). The tensile strength readings of each of the specimens were recorded when they failed. The results were statistically analyzed.

Fig. I:Measurement of Tensile Strength of Specimens.

SCANNING ELECTRON MICROSCOPE EVALUATION

The 2 specimens from each group prepared for SEM evaluation were sectioned and gold plated by a sputtering device and the specimens were observed with SEM at 50X magnification.

METHOD OF STATISTICAL ANALYSIS

The tensile strength of the specimens from Groups I to VII on failure was noted and tabulated. The results were averaged (mean + standard deviation) for each parameter and are presented in Tables and graphs below. A significance level of 95% ($P<0.05$) was considered. The statistical software SPSS (Statistical Package for Social Sciences) v 11.0 was used for data analysis using the following methods of statistical evaluation.

1. One Way Analysis of Variance (ANOVA): One way ANOVA is used to find out significant difference between many groups
2. Students unpaired t Test: t test is used to find significant difference between any 2 groups.

RESULTS

Effect of Different Chemical Impregnating Agents Tensile strength values indicate that Group VII (control group) has maximum tensile strength & Group VI has minimal tensile strength.(Table II)

Table .II Tensile strength values(in kg) for standard cotton cord by impregnation with different chemicals

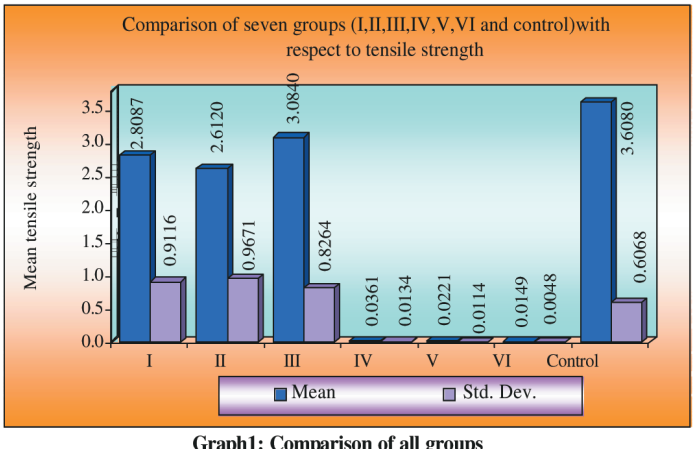
Groups	Mean	Std. Dev.	Standard Error	Confidence interval	
				-0.9500	0.9500
I	2.8087	0.9116	0.2354	2.3038	3.3135
II	2.6120	0.9671	0.2497	2.0765	3.1475
III	3.0840	0.8264	0.2134	2.6263	3.5417
IV	0.0361	0.0134	0.0035	0.0287	0.0436
V	0.0221	0.0114	0.0029	0.0157	0.0284
VI	0.0149	0.0048	0.0012	0.0122	0.0175
Control	3.6080	0.6068	0.1567	3.2720	3.9440

1. EFFECT OF AS IMPREGNATION IN GROUPS I, II & III.

When inter group comparisons were made between AS impregnated groups (Group I, II, III) and the control group (Group VII) to interpret the influence of chemical impregnation of specimens with AS, it is seen that the mean tensile strengths and their standard deviations were 2.8087 + 0.9116, 2.6120 + 0.9671, 3.0840 +0.8264 and 3.6080 + 0.6068 respectively. One Way ANOVA showed a statistically significant difference between the groups with P = 0.0122 (P < 0.05) indicating that F statistical value (F=3.9802, Degree of freedom = 3, 56) is statistically significant (Table III, Graph I).

Table.III Comparison of Groups[I,II,III(AS)and VII(control)]with respect to tensile stren by One-way ANOVA

SV	Degrees of freedom	Sum of squares	Mean sum of square	F-value	P-value	Significance
Between groups	3	8.41	2.80	3.9802	0.0122	
Within groups	56	39.44	0.70		<0.05	S
Total	59	47.85				



Graph1: Comparison of all groups

2. EFFECT OF FS IMPREGNATION IN GROUPS IV, V & VI.

When inter group comparisons were made between FS impregnated groups (Groups IV, V, VI) and the control group to interpret the influence of chemical impregnation of specimens with FS, it is seen that the mean tensile strengths and their standard deviations were 0.0361 + 0.0134, 0.0221 + 0.0114, 0.0149 + 0.0048 and 3.6080 + 0.6068 respectively. One Way ANOVA showed a statistically significant difference between the groups with P = 0.0000 (P < 0.05) indicating that F statistical value

Table. IV Comparison of Groups [IV,V,VI(FS) and VII(control)]with respect to tensile strength by One-way ANOVA

SV	Degree of freedom	Sum of squares	Mean sum of square	F-value	P-value	Significance
Between groups	3	144.48	48.16	522.7426	0.0000	
Within groups	56	5.16	0.09		<0.05	S
Total	59	149.64				

(F = 522.7426, Degree of freedom 3, 56) is highly significant (Table IV, Graph 1).

Comparison of AS and FS impregnated groups (Group I to VI) and the control group (Group VII) with respect to tensile strength by One Way ANOVA showed statistically significant difference between the groups with P = 0.0000 (P < 0.05) indicating that F statistical value (F = 99.5476, Degree of Freedom 6, 98) is highly significant (Table V). One Way ANOVA therefore rejects the null hypothesis of no difference in tensile strengths of the specimens, which means that there exists a significant

Table. V Comparison of six groups [(I,II,III,IV,V,VI) and VII(control)]with respect to tensile strength by one-way ANOVA

SV	Degree of freedom	Sum of squares	Mean sum of square	F-value	P-value	Significance
Between groups	6	240.43	40.07	99.5476	0.0000	
Within groups	98	39.45	0.40		<0.05	S
Total	104	279.88				

difference in tensile strength of specimens due to chemical impregnation and is not a chance occurrence.

EFFECT OF VARIATION IN CONCENTRATIONS OF CHEMICAL IMPREGNATING AGENTS

1. EFFECT OF VARIATION IN AS CONCENTRATION.

Pairwise comparison of groups I, II, III and VII using t test showed 't'

Table.VI Pair wise comparison of Groups [I,II,III(AS) and VII (control)] with respect to tensile strength by t-test

Group	Mean	SD	t-value	p-value	Significance
I	2.8087	0.9116	0.5731	0.5711	
II	2.6120	0.9671		>0.05	NS
I	2.8087	0.9116	-0.8666	0.3935	
III	3.0840	0.8264		>0.05	NS
II	2.6120	0.9671	-1.4370	0.1618	
III	3.0840	0.8264		>0.05	NS
I	2.8087	0.9116	-2.8270	0.0086	
Control	3.6080	0.6068		<0.05	S
II	2.6120	0.9671	-3.3788	0.0022	
Control	3.6080	0.6068		<0.05	S
III	3.0840	0.8264	-1.9794	0.0577	
Control	3.6080	0.6068		<0.05	S

and 'P' values at - 2.827 and 0.0086 between Group I and VII & - 3.3788 and 0.0022 between group II and control rejecting the hypothesis of equality of means at 5% level of significance ($P < 0.05$) implying that the means are statistically significant and there exists a difference in tensile strengths of cords by virtue of impregnation with AS. However comparison of means between Groups I and II, Groups I and III, Groups II and III, Groups III and control by 't' test showed no statistically significant difference in tensile strengths between each group implying that the variation in concentration of AS did not vary the tensile strength of cords significantly (Table VI Above).

2. EFFECT OF VARIATION IN FS CONCENTRATION.

Similarly pairwise comparison of Groups IV, V, VI and VII using 't' test (Table VII) showed statistically significant difference in tensile strengths between the groups thus implying that tensile strength of cords reduced significantly with the variation in FS concentrations.

Table.VII Pair wise comparison of Groups [IV,V,VI(FS) and VII(control)]with respec tensile strength by t-test

Group	Mean	SD	t-value	p-value	Significance
IV	0.0361	0.0134	3.0905	0.0045	
V	0.0221	0.0114		<0.05	S
IV	0.0361	0.0134	5.7733	0.0000	
VI	0.0149	0.0048		<0.05	S
V	0.0221	0.0114	2.2500	0.0325	
VI	0.0149	0.0048		<0.05	S
IV	0.0361	0.0134	-22.7929	0.0000	
Control	3.6080	0.6068		<0.05	S
V	0.0221	0.0114	-22.8842	0.0000	
Control	3.6080	0.6068		<0.05	S
VI	0.0149	0.0048	-22.9335	0.0000	
Control	3.6080	0.6068		<0.05	S

When pairwise comparison was carried out between AS and FS groups (I, II, III, IV, V, VI) using a t- test a statistically significant difference in the tensile strength between all the groups was seen.

SCANNING ELECTRON MICROSCOPIC EVALUATION

Evaluation of the effects of chemical impregnating agents and variations in their concentrations on the ultrastructure of the gingival retraction cords was done by scanning electron microscope at 50X and 500X. SEM analysis was done on specimens, which were not subjected to tensile loading. Certain morphological differences were apparent on visual evaluation of the SEM pictures. The images revealed that as compared to the Group VII specimens, specimens from all other groups showed comparatively greater degradation of strands thereby confirming the deleterious effect of chemical impregnating agents on these cords .

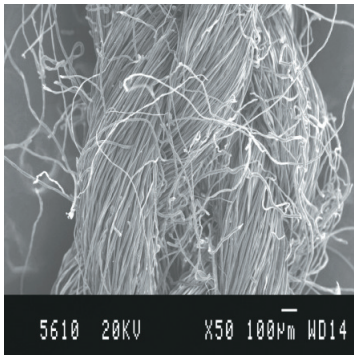


Fig. II: SEM Image of specimen from GroupVII at 50X

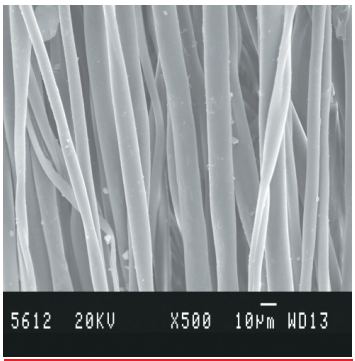


Fig.III: SEM Image of specimen from Group VII at 500 X

The control group specimens showed smooth continuous fibers. No presence of debris was evident. Distinct outline of the fibers could be made (Fig II, Fig III). The AS impregnated groups showed slight discontinuity along the length of fibers. Debris was present between the fibers and broken fibers were clearly visible (Fig IV, Fig V). In the FS impregnated groups severe discontinuity of fibers was evident. A greater amount of debris was present between the fibers. The outline of the fibers was indistinct as an increased number of broken fibers could be seen (Fig VI, VII). The amount of crystals deposited between the fibers increased as the concentration of AS and FS were increased.

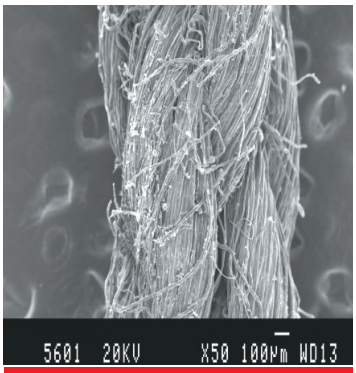


Fig.IV: SEM Image of specimen from Group III at 50 X

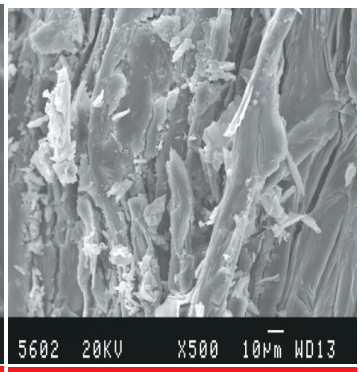


Fig. V: SEM Image of specimen from Group III at 500 X

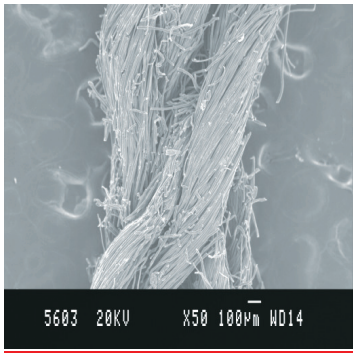


Fig. VI: SEM Image of Specimen from Group VI at 50 X

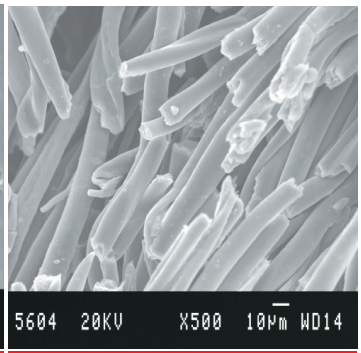


Fig. VII: SEM Image of Specimen from Group VII at 500 X

DISCUSSION

The success of Fixed Prosthodontic restorations is largely dependent upon the long term health and stability of the surrounding periodontal structures[26]. Full coverage restorations often require subgingival margins because of caries, esthetic demands, existing restorations, additional retention or other reasons [27,28], necessitating the need for proper exposure of finish lines so as to facilitate proper recording of the margin in impressions.

Even though this procedure of gingival retraction has its merits, it is quite technique sensitive and may result in injury to gingivae[19] varying from reversible to irreversible if not executed meticulously. The probability of tearing, shredding and fraying of retraction cords has been attributed mainly to their deficient tensile strength. Inadequate tensile strength may be either due to the effect of inherent mechanical structure (plain, twisted, knitted or braided) and chemical nature (cotton, nylon etc.) or by the action of the caustic chemical impregnating agent, used to control hemorrhage on the integrity of the cords.

Gingival Retraction cord has to be strong enough to bear the force of

cord manipulation, placement and removal from the sulcus for the purpose of gingival conditioning prior to impression making. The findings from the present study constitute one of the initial efforts to optimize this physical property of gingival retraction cords - the tensile strength. The lack of surplus information with respect to gingival retraction cords prevents meaningful comparisons with quantitative reports. The present research indicates certain cord features that could be worked upon to develop an ideal cord with a lower risk of suffering tears in the gingival sulcus and optimum finish line exposure. In turn, this should lead to decreased clinical time and minimize the risk of tissue damage. We emphasize that gingival retraction should not be at the expense of gingival health.

The most common primary medicament used by prosthodontist for finish line exposure is buffered aluminium chloride 55% followed by 23% FS[29], and hence was included in the present study.

Influence of Chemical Impregnating Agent

The mean tensile strength of the AS impregnated specimens showed 15 to 22% reduction when compared to the control (standard cotton cords) while that of FS impregnated ones showed 99% reduction. The reduction is much more pronounced with FS as compared to AS and is in agreement with findings from previous study[24] and is largely because of its chemical nature which is comparatively much more caustic than AS.

Based on the findings of this study it can be said that the impregnating chemicals produce a 'Degrading Effect' on the cords. It is obvious from SEM evaluation that with AS impregnated cords the degrading effect on the cotton thread was less. It is also apparent that the chemical AS per se did not disintegrate the cotton fibers to the extent as was seen with FS. Severe discontinuity in fibers seen in FS impregnated cords shows an obvious degradation effect of FS on the cords compared to control and AS impregnated cords. Dissolution of the fibers leading to increased number of broken fibers resulted in very low tensile strength. Most of the retraction agents have low pH and are acidic in nature. Dissolution of cotton fibers and decrease in the tensile strength of the cords can be correlated to the low pH of the chemicals used. As stated by Woody and Miller FS hydrolyzes in water to form sulfuric acid. This can damage the oral tissues and have a more profound effect than the etchants used for composite resin preparation. Action of sulfuric acid is not self limiting and its action is continuous until diluted. This reason could be attributed to further decrease in tensile strength of FS impregnated cords.

Various authors have reported irreversible injury to the sulcular epithelium during placement of gingival retraction cords into the gingival sulcus,[19, 25] and have outlined the type of chemical used for impregnating the cords, the length of time the cord is left in the sulcus and the force of packing as the primary cause of this irreversible injury[26]. From the present study the type of chemical used for impregnating the cords and the length of time the cords are left in the sulcus both can be suggested as interdependent factors as is the case with FS, where FS impregnation leads to such a drastic reduction in tensile strength that shreds of retraction cords are left behind into the sulcus due to tearing thereby prolonging the contact time between the cords and the sulcular epithelium.

INFLUENCE OF CONCENTRATION VARIATION

In the present study, amidst the AS impregnated groups the tensile strength non significantly reduced from Group III > Group I > Group II, even though the concentration of AS was greatest in Group III and least in Group I i.e. no linear variation was observed, however a general increment in tensile strength was observed with an increase in concentration of AS, probably the existence of a threshold effect of the chemical on the cords also cannot be ruled out and needs further evaluation. This was in stark contrast to the FS impregnated groups where the tensile strength reduced from Group VI < Group V < Group IV i.e. tensile strengths were inversely proportional to the concentration of FS . This is in agreement with other studies[30,31] which have also reported variation in gingival response to different concentrations of different chemical impregnating agents.

CONCLUSION

The present study was undertaken to evaluate the effect of various concentrations of different impregnating agents on the tensile strength and ultrastructure of gingival retraction cords. Based on the results and within limitations of the study the following conclusions can be drawn.

1. Impregnation of retraction cords with AS leads to significant reduction in tensile strength as compared to control groups.
2. Impregnation of retraction cords with FS leads to significant reduction in tensile strength as compared to control groups.
3. Increase in concentration of AS leads to a slight increase in tensile strength of retraction cords.
4. Increase in concentration of FS leads to a significant decrease in tensile strength of retraction cords.
5. SEM analysis of retraction cord fibers impregnated with AS exhibited slight discontinuity of the fibers along their length with visible broken fibers i.e. slight weakening of the cord ultrastructure and thus its integrity.
6. SEM analysis of retraction cord fibers impregnated with FS exhibited severe discontinuity of fibers along their length with a lot of visible debris and broken fibers i.e. severe weakening of the cord ultrastructure and thus its integrity.

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AUTHORS

1. Dr.Sakshi Madhok
   M.D.S. (Prosthodontics)
   Senior Resident,
   Department of Dentistry,
   Gajra Raja Medical College,
   Gwalior,M.P, India

2. Dr.K.Kumaraswamy
   Professor & Head
   Department of Prosthodontics
   AME's Dental College
   Raichur,Karnataka

3. Dr.Saksham Madhok
   Post Graduate Student (Orthodontics)
   MaharanaPratap Dental College and Research Centre,
   Putlighar Road
   Gwalior,M.P, India
   Mobile: 09584198983
   Email Id:drmadhok83@gmail.com

Correspondence Address

Dr.Sakshi Madhok
B-17, Samadhiya Colony, Taraganj,
Lashkar Gwalior-474001
Madhya Pradesh, India
Telephone: 0751-4067559
Email Id: sakshi_madhok@yahoo.co.in