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THE MORPHOLOGY OF ROOT SURFACE FOLLOWING ROOT CONDITIONING IN COMBINATION WITH ULTRASONIC IRRIGATION AS OBSERVED ON A SCANNING ELECTRON MICROSCOPE

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Abstract

The aim of this study was to compare the efficacy of ultrasonic irrigation in combination with root conditioning on the exposure of collagen fibrils in extracted human teeth using a scanning electron microscope.

Thirty periodontally healthy teeth were used in this study which was root planed and sectioned longitudinally to get sixty specimens which were included in six groups containing ten specimens each. The groups included two control and four experimental groups. The control groups received saline and ultrasonic irrigation respectively. The experimental groups received citric acid and tetracycline hydrochloride conditioning with saline and ultrasonic irrigation respectively. These specimens were examined under a scanning electron microscope and visualized for presence of exposed collagen fibrils.

The results showed a significant amount of exposed collagen fibers in the group which underwent ultrasonic irrigation in combination with root conditioning..

It can be concluded that ultrasonic irrigation before and after root conditioning improves exposure of collagen fibrils, which may be desirable for clinical success in periodontal regenerative therapy.

The clinical significance of this current study is that when root planing is done, it leaves behind a smear layer which may be detrimental for periodontal reattachment. Root conditioning with chemicals removes the smear layer without exposing the collagen fibers on the dentinal surface. This study shows that ultrasonic irrigation in combination with root conditioning removes the smear layer and exposes the collagen fibers on the root surface which may be desirable in the success of outcome of regenerative or periodontal plastic surgery.

Keywords: Ultrasonics, Root conditioning, Citric acid, Tetracycline, Collagen exposure. Scanning electron microscope.

INTRODUCTION

Periodontitis is characterized by inflammation of the various components of periodontium and produces substantial changes in the tooth and root surface which is then referred to as pathologically exposed. In a pathologically exposed root surface, the periodontal ligament fibers are destroyed by plaque induced inflammation, allowing down-growth of junctional epithelium. With loss of collagen, the root surface becomes hyper mineralized, plaque and calculus penetrates into the cementum/dentin of the root. Thus, the root surface becomes toxic and unsuitable for periodontal regeneration.1,2

In periodontics, regeneration implies the formation of new cementum, periodontal ligament and alveolar bone adjacent to a previously pathologically exposed root surface. Hence the attachment of connective tissue cells to the root surface of the teeth following periodontal surgery is a prerequisite for periodontal tissue regeneration.3,4

A critical step in periodontal regeneration is to alter the periodontitis affected root surface to make it a hospitable substrate to support and encourage migration, attachment, proliferation of periodontal connective tissue progenitor cells, or a process called as root biomodification.5

Root biomodification involves use of chemicals which results in complete removal of the smear layer, normally found after root planing. A number of chemicals have been proposed for the demineralization procedure including citric acid, tetracycline hydrochloride, EDTA and fibronectin. Of these citric acid and tetracycline hydrochloride had been extensively researched and used clinically because of higher tissue tolerance and easy storage.

Recent studies6,7 has been conducted to determine the influence of ultrasonic irrigation in combination with root conditioning. The result shows that ultrasonic irrigation before and after acid treatment removes the smear layer completely and could also result in improved exposure of collagen fibers. This exposed collagen fibres could result in splicing of the old and new collagen fibres and also help fibroblast attachment to the root surface.8

The purpose of the present study an attempt is made to compare the added efficacy of ultrasonic irrigation before and after acid demineralization on the exposure of collagen fibers in non diseased human root surfaces with plain acid conditioning alone.

METHODS AND MATERIALS

The material for the present In–vitro study consisted of thirty mandibular first premolar teeth in patients between the age groups of 15 to 25 years which were extracted due to orthodontic reasons. The teeth selected were free from calculus, dental caries, restorations, erosions, abrasions or periodontal diseases and were stored in a bottle containing normal saline6.

Then each of these premolars was root planed using a Gracey curette (Hu – Friedy No.

1-2) used under saline irrigation to remove cementum and expose the underlying dentine. The strokes were directed apico – coronally starting from the left side to the right side. The strokes begin from the apical area of the root terminating at the cemento – enamel junction.7

A high speed air rotor hand piece with copious water coolant was used to resect the crown at the cemento - enamel junction. Following the separation of the root from the crown, the apical third of the root was resected. The root surface was then sectioned bucco - lingually parallel to its long axis to yield two specimens.9 The specimens was washed properly with saline and stored in sterile bottles containing saline until the further procedures.6

Thus sixty specimens were obtained which were grouped initially into two groups (saline group and ultrasonic group) and then into three subgroups containing ten specimens each. Thus, there were six subgroups totally containing ten specimens each.7Each group had one control group receiving only saline or ultrasonic irrigation and two experimental groups receiving saline or ultrasonic irrigation before and after citric acid or tetracycline hydrochloride conditioning. Group SI (control) which received only saline irrigation. The irrigation was performed using 10 ml syringe with adequate pressure for 3 minutes. Group SII and SIII specimens received saline irrigation followed by citric acid or tetracycline hydrochloride immersion respectively for 3 minutes and again the samples were treated with saline irrigation. Group UI (control) received only ultrasonic irrigation. Mini Piezon EMS ultrasonic scaler with universal tip was used under medium power setting. The irrigation was performed by gently working the tip on the root surface for 3 minutes. Group UII and UIII specimens receiving ultrasonic irrigation followed by citric acid or tetracycline hydrochloride immersion respectively for 3 minutes and again irrigated with ultrasonic scaler in the same manner mentioned above.

After the experimental procedures were completed, all the specimens were fixed with 4% formaldehyde in 0.2 M phosphate buffer (Ph = 7.2) at room temperature for 24 hours. Following fixation, the specimens were dehydrated using an ascending series of graded ethyl alcohol with the following concentrations: 33%, 50%, 67%, 95% &100% for 10 minutes at each concentration. The specimens were then air dried overnight.10

The specimens were mounted on S.E.M. stubs and then were coated with gold using the gold sputter coater. The specimens were examined and photographed at magnification x 2000. A suitable field was selected in the specimen and was copied and stored in a computer. A 4 × 5inch print of the SEM was made. The surface characteristics of the dentinal specimen were evaluated descriptively for the presence of exposed collagen fibrils.

Chi square test was used to determine if there are any significant differences in the groups i.e., saline group and ultrasonic group and in between the groups.

RESULTS

Group SI (control) did not show any exposure of collagen fibers. There is presence of a smear layer too covering the root surface (Fig I). Group SII and SIII had no smear layer and had many open patent dentinal tubules of varying sizes and shapes. The lumen was rounded, cylindrical and elliptical in shape. There was collagen fiber exposure in few of the samples (Fig II & III).

Group UI (control) exhibited partially exposed dentinal tubules with a absence of smear layer. Collagen fiber exposure was not evident (Fig IV). Group UII and UIII also showed an absence of smear layer and exhibited the presence of indifferent fiber plexus formed by collagen fibers. Areas of dentinal tubular exposure were evident. There was a maximum exposure of collagen fibers here (Fig V & VI).

Chi square test indicated non-significant difference for groups SI with SII ($p>0.08$), SI with SIII ($p>0.17$), SII with SIII ($p>0.82$) and UII with UII ($p>0.59$). There was a significant difference for chi square test performed with group SII with UII (<0.01) and

SIII with UIII (<0.01). A highly significant difference for chi square test was found on comparison of groups UI with UII (p<0.001) and UI with UIII (p<0.001). Chi square test could not be performed on group SI with UI as three of the values were zero.

The overall results suggest that the ultrasonic treated group has more collagen fiber exposure when compared to the saline group.

Fig I: *Saline irrigated specimen showing a smear layer.*

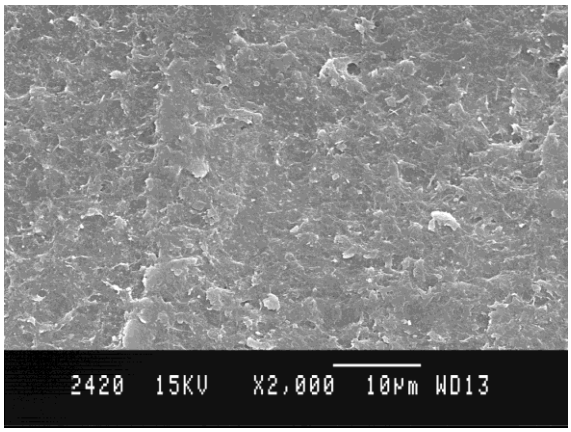


Fig II: *Ultrasonic irrigated specimen showing a partially removed smear layer.*

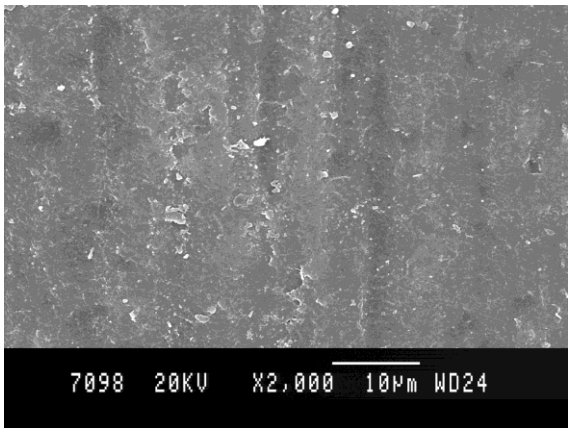


Fig III& IV:Citric acidand tetracycline hydrochloride treated and saline irrigated specimen showing complete removal of smear layer with exposed dentinal tubules. Partial exposures of collagen fibers are also seen.

Figure 3

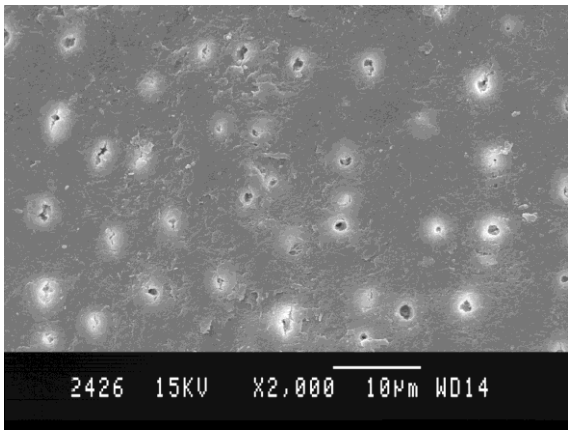


Figure 4

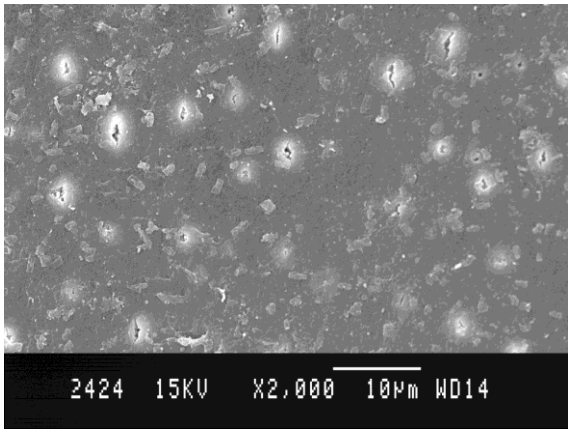


Fig V& VI: Citric acidand tetracycline hydrochloride treated and ultrasonic irrigated specimen showing complete removal of smear layer. More exposure of collagen fibers are seen as compared to the saline irrigated groups.

Figure 5

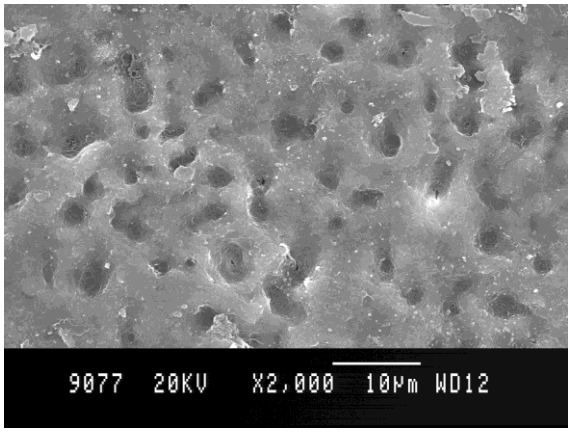


Figure 6

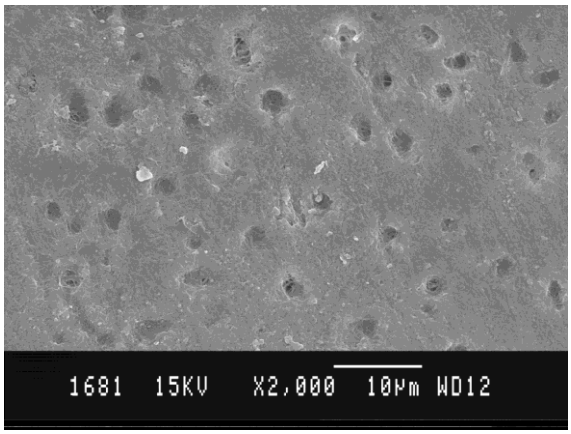


Table I *Root surface characteristics after saline rinsing and ultrasonic irrigation*

Group	**Root treatment**	**Exposed fibril***		
		+	±	-
Group SI	**Saline irrigation only**	**0/10**	**0/10**	**10/10**
Group SII	**Saline + citric acid + saline**	**2/10**	**2/10**	**6/10**
Group SIII	**Saline + tetracycline hydrochloride + saline**	**1/10**	**2/10**	**7/10**
Group UI	**Ultrasonic irrigation only**	**0/10**	**1/10**	**9/10**
Group SII	**Ultrasonic + citric acid + ultrasonic**	**8/10**	**2/10**	**0/10**
GroupSII	**Ultrasonic + tetracycline hydrochloride + ultrasonic**	**7/10**	**2/10**	**1/10**

Table – I: Root surface characteristics after saline rinsing and ultrasonic irrigation in combination with citric acid or tetracycline hydrochloride conditioning.

* = Number of specimen exhibiting exposed fibrils or grinding debris/

Total number of specimen

+ = Present; ± = Rarely present; - = absent:

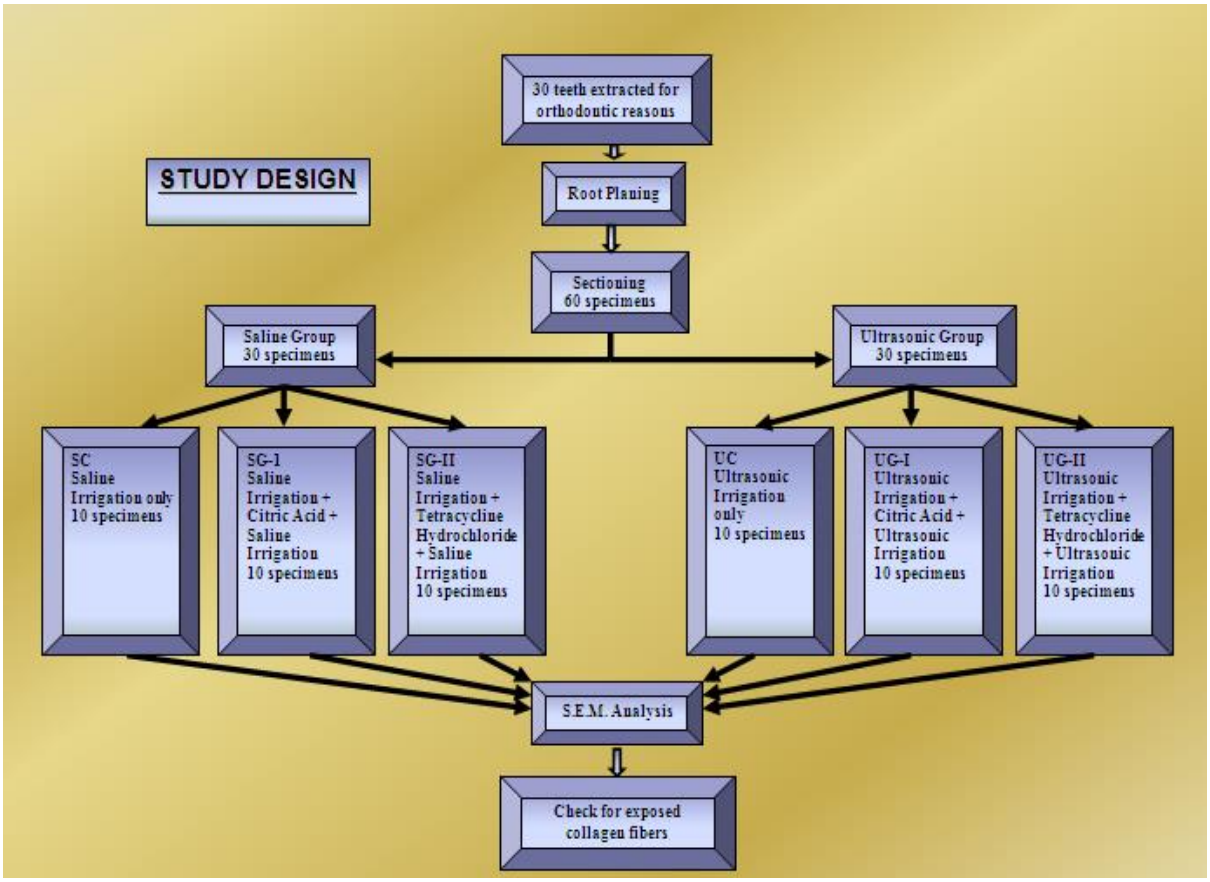
TABLE II *FOR EXPOSED COLLAGEN FIBERS*

Comparison groups	**Chi square (χ^2) value**	**p value**	**Level of significance**
SI vs. SII	5.00	> 0.08	Not Significant
SI vs. SIII	3.53	> 0.17	Not Significant
SII vs. SIII	0.41	> 0.82	Not Significant
UI vs. UII	17.33	< 0.001	Highly Significant
UI vs. UIII	13.73	< 0.001	Highly Significant
UII vs. UIII	1.07	> 0.59	Not Significant
SI vs UI	¶	¶	¶
SII vs. UII	9.60	< 0.01	Significant
SIII vs. UIII	9.00	< 0.01	Significant

¶ = Chi Square test not possible because three of the values are zero

Table – II: Intra group statistical analysisfor saline and ultrasonicgroup and intergroup statistical analysis between saline and ultrasonic group.

Chart – I: *The study design.*



DISCUSSION

The ultimate goal of periodontal therapy is predictable regeneration of a periodontium previously destroyed by periodontitis. A major inhibiting factor for predictable regeneration appears to be the nature of the periodontitis affected root surface. The exposed root surface associated with periodontitis undergoes substantial alterations, that is, the fiber attachment system is destroyed resulting in a denuded and contaminated root surface.2,11 Hence the critical step in periodontal regeneration is to alter this contaminated root surface so as to make it more suitable for periodontal regeneration. Mechanical and chemical methods had been proposed to remove the root surface deposits.5

Scaling and root planing can remove the hard and soft deposits, the diseased cementum and dentinal surface but leaves behind the smear layer.12 Hence acids like citric acid and tetracycline hydrochloride have been used to remove this smear layer left behind by mechanical instrumentation and to expose the intrinsic collagen of the root dentin.4,5,9,13,14,15,16 Acid treatment of periodontally diseased teeth however resulted in unsuccessful clinical results.14,17,18,19,20 Possible reasons for this failure could be inconsistent methodology with respect to application time, technique, as well as solution pH or concentration21. Another reason for no apparent beneficial effects of acid demineralization could be inadequate treatment before and after acid application. This was again supported by previous studies14,17,18,19,20,22 where they have demonstrated unsuccessful results due to inadequate rinsing after acid application wherein the root surfaces were only rinsed with saline solution. Recent studies have showed that ultrasonic irrigation before and after acid application improves the exposure of collagen fibrils, which in turn may be desirable for clinical success6,7. They reported that, ultrasonic irrigation before acid application

disturbs the smear layer and hence the acid penetration could be deeper and ultrasonic irrigation after acid application exposes a dense, fibrous network of collagen.

In a study where citric acid and tetracycline hydrochloride were compared as root conditioners, it was reported that citric acid produced better results then tetracycline hydrochloride23. This is in contrary to our study results where both citric acid and tetracycline hydrochloride were equally effective in exposing collagen fibers. ($p > 0.62$) The reason for this difference could be because we in our study used chemically pure tetracycline hydrochloride whereas many other studies have used tetracycline hydrochloride directly from the capsule.

The results of this in vitro study indicated that demineralization of root dentin supplemented by ultrasonic irrigation before and after the application of the acid, is effective in removing grinding debris and exposing collagen fibrils. However further experimental and clinical studies are needed to evaluate the beneficial effects of citric acid or tetracycline hydrochloride supplemented by ultrasonic irrigation, which may play a role in facilitating connective tissue attachment.

CONCLUSION

The following conclusions were drawn:

1) Saline irrigation alone or saline irrigation in combination with citric acid or tetracycline hydrochloride solutions was ineffective in exposing collagen fibers.

2) Ultrasonic irrigation before and after acid treatment exposes collagen fibers.

3) When saline group and ultrasonic group were compared, ultrasonic group showed better exposure of collagen fibers.

4) Highly significant difference was found for exposed collagen fibers between the ultrasonic irrigation alone and ultrasonic irrigation before and after acid treatment.

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