Scanning Electron Microscope (SEM) Study to Compare Manual Scaling and Root Planing With and Without Magnification: An In-Vitro Study

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In

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By

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TABLE OF CONTENT

S. No.	Contents	Page No.

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

LIST OF ABBREVIATIONS

et al.	et alia (And others)
FESEM	Field emission scanning electron microscope
LDD	Local Drug Delivery
LPS	Lipopolysaccharides
LTSI	Loss of Tooth Substance Index
Mm	Millimetre
Nm	Nanometre
NSPT	Non-Surgical Periodontal Therapy
Pa	Pascal
Pd	Palladium
Pt	Platinum
Pvt.Ltd.	Private and limited
RCI	Remaining Calculus Index
RTLSI	Roughness Loss of Tooth Substance Index
SEM	Scanning Electron Microscopy
SRP	Scaling and Root Planing

LIST OF ANNEXURES

ABSTRACT

A vast array of periodontal pathogens initiate the dysfunctionality of the immune and inflammatory responses in the host periodontal tissues, causing bone and periodontal attachment loss. The key etiologic agents in periodontitis are bacterial plaque and calculus and the removal of all deposits from tooth surfaces is the most essential component of periodontal treatment. This can be done manually or with ultrasonic scalers. The incorporation of magnifying tools to visualize the area to be treated helps in increasing the efficacy of the treatment. So, our study was carried out to compare scaling and root planing done under magnification to scaling and root planing done under naked eye that was assessed by Scanning electron microscope (SEM) findings. The objective of the study was to compare and assess Tooth surface roughness, Remaining calculus and Loss of tooth structure with and without magnification. The study was done on extracted teeth following inclusion and exclusion criteria. The extracted teeth were mounted (randomly and equally assigned to Group II and Group III). SRP was done manually. Afterwards the teeth specimen was sagittally sectioned to be viewed for SEM analysis. Remaining calculus Index, Loss of Tooth Substance, Tooth Surface Roughness and the presence or absence of smear layer were the criteria that were assessed. From the present study it was concluded that SRP when done under magnification with the help of magnifying loupes noticeably increased the success of the procedure. It was clearly evident by complete removal of even tiny flecks of calculus which otherwise, might at times be left behind and further become a nidus of plaque accumulation. With more precise strokes, the calculus removal was easier. Since, more precise and less number of stroke are carried out, there were lesser

instrument marks or scratches on the teeth; lesser amount of overall loss of tooth structure as it was supported by SEM analysis of the sectioned teeth. It is possible to assume that magnification proves to be better, precise, controlled, less invasive or destructive modality when compared to carrying out the procedures under naked eye. As much as possible the dental operator must use the magnifying loupes in their clinical practices to enhance both operator and patient treatment outcomes. Further studies need to be done to understand quantity and quality of strokes so as to prevent overzealous instrumentation. Clinically and histologically, SRP under magnification with magnifying loupes leads to positive outcomes.

A vast array of periodontal pathogens initiate the dysfunctionality of the immune and inflammatory responses in the host periodontal tissues, causing bone and periodontal attachment loss[1].The key etiologic agents in periodontitis are bacterial plaque and calculus and the removal of all deposits from tooth surfaces is the most essential component of periodontal treatment. NSPT (Non-Surgical Periodontal Therapy) is the major treatment modality that is rendered and the main objective is to restore gingival health and prevent further loss of periodontal structures. This is done as to produce a shift in the composition of subgingival ecosystem from gram negative anaerobes to gram positive facultative bacteria compatible with health[2]. This is likely by reducing the amount of tooth associated biofilms and their biological products, such as endotoxins, antigens, enzymes and other tissue irritating factors. Non-Surgical Periodontal Therapy (NSPT) includes SRP and/or use of Local Drug Delivery (LDD) and/or use of adjunctive antimicrobials depending upon the severity of the periodontal disease. SRP can be done with manual and ultrasonic instruments.

It has also been seen that tooth surface tends to become rough along with sometimes loss of tooth substance that occurs both due to the action of plaque micro-organisms and the SRP procedures itself, at times leading to dentinal tubule exposure and tooth hypersensitivity[1]. Hence, the purpose of periodontal therapy should be such that it leads to lower the levels of pathogenic micro-organisms and not adversely affect the tooth structure.

At times complete elimination of deposits from the tooth surface, especially inaccessible areas is slightly difficult to achieve under naked eye. For this reason,

magnification of the area to be instrumented can be done. Magnification enhances the visual acuity, illumination and intensification of the operator to attain better clinical outcomes. This is done by the use of magnifying loupes and surgical operating microscopes. Loupes are the most frequently used magnification system in dentistry. It comprises of two monocular microscopes, with side by side lenses, angled to focus on an object to form magnified imagesranging from 1.5 to 10 magnifications. According to Tascheiri S et al. magnification instruments are useful for both clinicians and patients, in terms of ergonomics, vision, rate of successful treatment and treatment time[3].

Scanning Electron Microscopy (SEM) has been a useful tool in research. It is one of the common methods for imaging the microstructure and morphology of the materials and structures. SEM allows the visualization of images at high magnification of 50 to 10000 magnification and above. In this technique when a focused beam of electrons strike surface topography of the specimen, it produces variety of waves (the waves produced depends upon the type of the specimen). The feedback is collected by the detector. Thus, it brings out the detailed surface topography of specimen. Regions with lower atomic number appear darker than with the higher atomic number.

Hence, in the above study, an attempt has been made to analyze the morphological changes of the roughness of tooth structure, loss of tooth substance and remaining calculus on the tooth surface following SRP procedures under magnification and naked eye, assessed by means of SEM studies in order to find out whether a co-relation exists between the mentioned morphological changes of teeth under and without magnification.

AIM AND OBJECTIVES

AIM:

To compare scaling and root planing done under magnification to scaling and root planing done under naked eye assessed by Scanning electron microscope (SEM) findings.

OBJECTIVES:

1. To assess and compare the remaining calculus on the tooth surface by Remaining Calculus Index (RCI) given by Meyer and Lie in 1977[4].

2. To assess loss of tooth substance by Loss of Tooth Substance Index (LTSI) given by Meyer and Lie in 1977[4].

3. To assess roughness loss of tooth substance by Roughness Loss of Tooth Index (RLTSI) given by Meyer and Lie in 1977[5].

4. To assess presence or absence of smear layer under SEM[6].

REVIEW OF LITERATURE

Meyer K et al. (1977)[4] In this study amount of calculus removed and the amount of tooth substance lost after scaling and root planning (SRP) with the use of hand curette, ultrasonic scaler, rotating diamond and RotoPro® points was examined and studied under Scanning Electron Microscopy (SEM). Root surfaces of mandibular incisors positioned in a jaw model and have been cleaned of adhering deposits, photographed under SEM and evaluated at a high magnification by three examiners. The results revealed that most calculus was removed following diamond instrumentation, while the ultrasonic curette became least efficient. Hand curette, ultrasonic curette and roto-seasoned eliminated almost same amount of tooth substance whereas the diamond removed substantially extra than the others. The diamond regularly eliminated all the cementum and left deep instrumental marks within the dentin.

Eisuke Fukazawa et al. (1994)[7] The primary goal of this research was toto see if gingival fibroblasts adhere to curetted cemental surfaces in periodontitis-affected human teeth. A total of ten periodontally affected human teeth were used in the study. Five teeth had their surface cementum removed by mild mechanical curettage, while the remaining five teeth had no root treatment. The roots of all teeth were then sliced lengthwise. The prepared slabs were then seeded with human gingival fibroblasts (HGF) in 35 mm culture dishes and processed for transmission electron microscopy (TEM) after 4 weeks. The non-curetted cemental surfaces that failed to attach had flattened HGF, according to TEM results. Between the HGF and cemental surfaces, no fibrillar material was seen. HGF that developed on already curetted cementum, on the

other hand, had the usual shape of healthy, functioning fibroblasts. Both newly produced fibrillar material and collagen fibrils were seen, and both appeared to be directed toward the curetted cemental surface. The findings clearly imply that removing the superficial cementum first by mechanical curettage might promote better cellular attachment on regions of the root surface previously affected by periodontitis.

Knut Lekness et al. (1996)[8] The goal of this study was to see how gingival tissues reacted to plaque buildup on root surfaces that had been instrumented with diamond and curettes. In 5 beagle dogs, deep periodontal abnormalities were seen on the buccal surfaces of mandibular and maxillary canine teeth. A flame-shaped, fine-grained, spinning diamond point or a sharp curette were used to instrument the root surfaces. Tissue blocks of the experimental locations, comprising teeth, alveolar bone, and gingival tissues, were obtained when the dogs were killed. At three levels, the gingival soft tissue was treated for histomorphometric analysis. There were no changes in epithelium and connective tissue area measurements between the two instruments. Inflammatory cells (IC) were found in greater proportions in junctional epithelium (JE) cell point counts in specimens confronting diamond than in curette-instrumented defects. For both instrumentations, a greater proportion of IC was found in the coronal compared to the apical side of the JE. For both non-infiltrated and infiltrated connective tissue, there was a substantial variation in IC density between instrumentations. Overall, the findings show that the kind of subgingival root instrumentations has a substantial impact on gingival inflammatory responses, most likely via affecting subgingival plaque development.

Cadosch J et al. (2003)[9] This research was performed to evaluate between the number of standardized scaling strokes and reducing the amount of endotoxin from the

root surface. Scaling and root planing (SRP) of twenty-four extracted human caries-free single rooted teeth with at least 5 mm attachment loss was done. Standardized force instrumentation was applied using a force-measuring currette. Fifty working strokes were applied to every region of interest where subgingival calculus was present. Forces applied were recorded. The force recordings were converted from Millivolts into Newtons (N). After every unit of 5 strokes, presence of calculus was evaluated and scaling debris was collected. Endotoxin concentration was determined in the debris samples. The endotoxin values for strokes 1–5 were statistically significantly greater than the values from all other stroke intervals. Complete calculus removal occurred after a mean of 9.3 strokes. The endotoxin concentration reached a minimal level with concentrations of 0.03–0.306 EU/ml after calculus removal was complete. These findings suggest that completion of calculus removal coincides with endotoxin levels associated with clinically healthy teeth.

Ahmad Moghare et al. (2010)[10] The purpose of this study was to evaluate the efficacy of subgingival scaling and root planing using an erbium: yttrium, aluminium, garnet (Er:YAG) laser vs manual instrumentation. Hand instrumentation or Er:YAG laser irradiation were used to treat the mesial and distal surfaces of 15 periodontal loosed removed teeth at random. Three observers used scanning electron microscopy at magnifications of 50 x and 400 x to assess the morphology of the root surface. The rate of remaining roughness on treated root surfaces in two groups of hand tools and Er:YAG laser exhibited a significant difference as a result of this setting: Surface roughness was higher in the Er:YAG laser group than in the hand instruments group.

Preeti M et al. (2012)[11] They compared the amount of calculus left following root planing using a Gracey curette, an ultrasonic device, and a Desmo Clean™ to the

amount of tooth material lost and the roughness of the root surface. SEM was used to analyse 36 extracted mandibular incisors. The alterations were measured using three indices: the remaining calculus index (RCI), the loss of tooth substance index (LTSI), and the roughness loss of tooth substance index (RLTSI). Each group was used to treat 12 samples. It was also reported how long instrumentation took. There were no significant differences between the three groups on the RCI and LTSI. Slimline™ and hand curette, as well as Slimline™ and Desmo- Clean™, demonstrated a substantial difference in RLTSI. For RCI, LTSI, and RLTSI, Slimline™ had the lowest mean scores. As a result, even though the difference was not statistically significant, the Slimline™ insert outperformed the other techniques in terms of index scores and instrumentation time.

Sharmila V et al.(2012)[12] This study was done under Scanning Electron Microscopy, the morphological features of root surfaces following application of Carisolv gel in conjunction with scaling and root planing were examined. Sixty removed human teeth with periodontal disease were randomly assigned to one of four groups: 1.Scaling and root planing (SRP) alone, 2.Passive topical Carisolv + SRP application, 3.Active topical Carisolv + SRP application and 4.Numerous Carisolv + SRP applications. Carisolv gel was applied to the root surfaces for 30 seconds, then Gracey curettes were used to scale and plan the roots in 30 strokes. The sole exception was group 4, which had its roots instrumented until they were smooth, rigid, and glass-like. SEM was used to examine all of the specimens further. The smear layer was significantly reduced when several applications of Carisolv with SRP were done compared to a single application of the gel. The Carisolv gel failed to entirely eliminate the smear layer, even after a single application. The Carisolv gel caused alterations in

the root morphology of periodontally damaged teeth only when it was administered actively and numerous times, according to this study.

Mithul kumar M et al. (2013)[13] The goal of the study was to see if erbium doped: Yttrium aluminium garnet (Er:YAG) laser scaling and root planing could be used alone or in combination with hand and ultrasonic equipment. A total of 75 single-rooted teeth with periodontal involvement were collected. The teeth were split into five treatment groups, each with 15 teeth: Hand scaling, ultrasonic scaling, Er:YAG laser scaling, hand + Er:YAG laser scaling, and ultrasonic scaling + Er:YAG laser scaling are all options. SEM was used to analyse specimens, and images were reviewed by three examiners who were not aware of the project. Remaining calculus index, loss of tooth substance index, roughness loss of tooth substance index, presence or absence of smear layer, thermal damage, and any other morphological damage were among the parameters examined. In compared to the other test groups, Er:YAG laser treated specimens were equally efficient in removing calculus, although tooth substance loss and surface roughness were higher. When compared to other groups with various parameters, ultrasonic treated specimens had superior outcomes. The existence of a smear layer was more noticeable in the hand and ultrasonic groups, however. Thermal damage and morphological change were seen in very few laser-treated specimens.

Rajana M et al.(2013)[14] Using Magnifying Loupes (ML) and a dental operating microscope (DOM), a study was conducted to determine the efficacy of scaling and root planing (SRP). A total of 90 human teeth were randomly assigned to three treatment groups from 18 individuals with widespread chronic severe periodontitis who were between the ages of 25 and 65. SRP was done without magnification in Group 1. Group 2-SRP with ML and Group 3-SRP with DOM. Following extractions, samples

were prepared for atomic force microscopy, scanning electron microscopy, and elemental analysis using energy dispersive X-ray analysis to assess surface topography, the existence of a smear layer, and debris. The most successful approach for SRP was Group 3, whereas Group 1 was the least effective. The effectiveness of supragingival and subgingival SRP is greatly increased when using magnification equipment.

Rosales-Leal JI et al. (2014)[15] The researchers wanted to see how untreated single-rooted teeth looked after being planed in vivo with a curette, a piezoelectric ultrasonic (PU) scraper, or a vertically oscillating ultrasonic (VOU) scraper.44 single-rooted teeth were randomly allocated to one of four groups in a randomised experimental study of 19 patients: no treatment, manual root planing with a curette, with a PU scraper and with a VOU scraper. The teeth were removed after treatment and the roughness parameters arithmetic average height, root-mean-square roughness, maximum height of peaks, maximum depth of valleys, absolute height, skewness, and kurtosis were measured in 124 observations using white-light confocal microscopy. After each treatment, the roughness values of arithmetic average height and root-mean-square roughness were equal and lower than after no treatment. The VOU group had a shorter absolute height than the untreated and PU groups. After the three treatments, the surface morphology was comparable and less uneven than in the control group. All treatment groups had identical values for the remaining roughness measures. Both ultrasonic devices minimise roughness, resulting in a topography that is comparable to that seen following manual instrumentation with a curette, and they appear to be a viable alternative.

Robert Antonio A et al.(2017)[16] The goal of this study was to assess the cutting edges of Gracey curettes after manufacture and resharpening utilising a variety of

methods. Scanning electron microscopy was used to assess the cutting edges of 41 new #5-6 stainless steel Gracey curettes. Micrographs were used to assess the cutting edge quality blindly by a calibrated tester. A Kruskal Wallis test and nonparametric two-way multiple comparisons were used to evaluate the data. The results showed that different sharpening methods had considerably varied effects on cutting edge sharpness.

Abhay A Nawathe et al.(2017)[17] The goal of this study was to compare the effects of using QMix® and SofScale as an adjuvant to scaling and root planing (SRP) on periodontally damaged root surfaces using a scanning electron microscope (SEM). This study evaluated QMix® and SofScale as an adjuvant to SRP on periodontally damaged root surfaces under SEM in a single-blinded in vitro investigation. The total of rankings for QMix® was 306.50, whereas SofScale was 513.50. When compared to the SofScale group, the QMix® group exhibited statistically significant results (P = 0.004). A comparison revealed that QMix® had considerably superior smear layer reactivity.

D Fidyawati et al. (2017)[18] The goal of this research is to look at the smear layer on the root surface that has been conditioned with 2.1 percent minocycline HCl ointment (Periocline) and a 24 percent EDTA gel (Prefgel). A total of ten human teeth with chronic periodontitis that needed to be extracted were collected and root planed. The teeth were cut into thirds in the cervical region, yielding 30 samples that were split into three groups: minocycline ointment, 24 percent EDTA gel, and saline as a control. A scanning electron microscope was used to analyse the samples. There were no significant changes in smear layer levels between the minocycline and EDTA groups. When the minocycline and EDTA groups were compared to the control group, there were significant variations in the level of smear layer following root surface treatment.

Dadwal A et al.(2018)[19] In thisstudy, 30 extracted teeth with periodontal problems were chosen for this investigation. Three test groups were formed. Scaling and root planing were done with magnification loupes in test Group I and naked eyes in test Group II. No procedure was carried out in control Group III. Scanning Electron Microscope analysis was performed on the samples. The results revealed that Test Group II with magnification loupes had less residual calculus, a smoother surface, and less cementum layer loss than Test Group I and III.

Maritato M et al. (2018)[20] The goal of this study was to look at the changes in morphology and surface roughness in dental root samples after periodontal scaling using a hand curette, piezoelectric ultrasonic devices, or a combination of these. A total of twenty-four monoradicular teeth were removed due to periodontal disease and were separated into four groups: Piezoelectric ultrasonic scaler Piezon® Master 400 was used on Group A; piezoelectric ultrasonic scaler PiezoSmart® was used on Group B; Gracey curette 7/8 was used on Group C; and a combination technique of piezoelectric ultrasonic scaler Piezon® Master 400 and Gracey curette 7/8 was used on Group AC. A white light interferometer and scanning electron microscopy (SEM) were used to examine the treated samples. Roughness study indicated substantial surface changes in Group C, whereas samples treated with the combination method (Group AC) exhibited a smaller but still significant change, and samples treated with piezoelectric ultrasonic devices (Group A and B) had the lowest roughness values. There was a substantial difference between groups C and B. SEM research validated the roughness study, indicating non-homogeneous surfaces in Group C, whereas the other groups showed less morphological changes. When compared to piezoelectric ultrasonic devices, hand curettes appear to have a significant influence on surface integrity.

Sonika S et al. (2019)[21] A total of 10 teeth with grade II and III mobility in patients with chronic periodontitis who were scheduled for extraction were included in this research. The specimens were split into two groups: Group 1 and Group 2. Gracey curettes were used in both groups for scaling and root planning (SRP). During SRP, Group I utilised a microsurgical technique (x2.5 magnification compound loupe) while Group II used a conventional approach (naked eye). Teeth were removed, sectioned, and examined for the existence of any remaining calculus using a stereomicroscope after scaling and root planing. They discovered that teeth in Group 1 treated with a magnification loupe had less residual calculus than teeth in Group 2 treated without magnification.

S Varghese et al.(2019)[22] In this study, they used a scanning electron microscope to examine the morphological features of the root surfaces following scaling and root planing with curettes with a microscope (Group 3), dental loupes (Group 2), and naked eye (Group 1). Each Group received extracted teeth at random. Between the groups, the presence or absence of a smear layer, the remaining calculus index (RCI), and the roughness and loss of tooth substance index (RLTSI) were compared. Under an 12x magnification microscope, SRP was shown to give a smoother root surface with almost no smear layer and calculus.

Abhishek S et al. (2019)[23] In this study, following instrumentation using curettes as well as an ultrasonic perio mini-tip, an attempt was made to compare and assess the micro-topography of the root surface under a scanning electron microscope. Hand instrumentation with a curette and 150 strokes per surface provided the smoothest, cleanest, calculus-free, and most uniform root surface topography when compared to other SEM photomicrographs. 100 strokes of the curette seemed to achieve

the same effect as 45 seconds of ultrasonic instrumentation. As assessed by an eye inspection and a comparison of SEM photomicrographs. When comparing root surfaces treated with ultrasonic perio mini-tips to root surfaces treated with curettes, the amount of residual calculus flecks and smear layer embedded in cementum was higher in surfaces treated with ultrasonic perio mini-tips.

Sruthy N et al.(2019)[24] The purpose of this study was to compare the efficiency of two piezoelectric scalers and manual instrumentation in eliminating calculus, tooth material loss, and root surface roughness. Ninety periodontally impaired teeth, which were non-carious, non-restored, and single-rooted, were removed and utilised in the study. Scaling and root planing were performed using two piezoelectric scalers (Piezoelectric scaler A & Piezoelectric scaler B and Gracey curette, respectively). Scanning Electron Microscopy (SEM) was used to investigate the subjects, and profilometric analysis was used to measure the root surface roughness created by these three equipment. With piezoelectric scalers, the remaining calculus index (RCI) was shown to be the least effective in eliminating calculus in differentiation. In comparison to hand tools, SEM and profilometric tests revealed that Piezoelectric scaler A and B generated reduced root surface roughness. Piezoelectric tools had a smoother root surface than manual instruments and were more successful at removing calculus.

Amit G et al.(2019)[25] The goal of this study was to compare and contrast the effectiveness of Slimline ultrasonic inserts with manual tools in removing subgingival calculus. After scale and root planing, the morphology of root surfaces was studied using a scanning electron microscope. Thirty-six posterior teeth from patients with chronic periodontitis and a dismal prognosis were removed after SRP. Twelve teeth were put in each group, and SRP was performed to the depth of the pocket, and the

results were divided into three groups based on the instrument used: Group 1- Gracey curette 11/12 and 13/14, Group 2- Gracey curette 15/16 and 17/18, and Group 3- Ultrasonic Slimline inserts.The removed teeth were then examined using stereomicroscopy and a scanning electron microscope (SEM). When comparing Gracey curettes 11/12 & 13/14 and Gracey curettes 15/16 &17/18, it was discovered that the quantity of residual calculus present was lowest when using ultrasonic Slimline inserts, and that the difference between the two groups of Gracey curettes was not significant. The use of ultrasonic scalers with Slimline inserts during subgingival SRP is more successful than manual tools, according to the findings.

Mahmoud Al Ankity et al.(2020)[26] The research looked at the effects of hand and ultrasonic tools composed of stainless and titanium metals on the enamel surface layer. Forty removed premolars were employed in this in-vitro investigation, which were uniformly split among four groups. Group A had ultrasonic scaling with a stainless steel tip, group B had titanium tip ultrasonic scaling, group C had stainless steel tip hand scaling, and group D had titanium tip hand scaling. The enamel surface topography was examined using scanning electron microscopy (SEM). In group D, SEM exhibited severe roughness and disastrous alterations on the enamel surface, but the topography of the enamel surface in other groups was less altered. Atomic Force Microscopy revealed that hand instruments with titanium curettes had the largest mean surface roughness difference, whereas ultrasonic tips with stainless-steel tips had the lowest mean surface roughness difference. Scaling using ultrasonic stainless steel tips leaves the tooth surface topography with the least degree of roughness and damage.

Muhammed Bedir Mahiroglu et al. (2020)[27] The purpose of this study was to examine root surface wear and roughness on removed human mandibular incisor teeth

caused by professional dental hygiene tools such as ultrasonic dental scalers, rubber cups, and nylon bristle brushes. Eighty teeth were divided into eight groups based on the type of scaler used (Ma = Magnetostrictive, Pi = Piezoelectric), the degree of power (M = Medium, F = Full), and the angulation (0 and 45).The forty specimens were then split into two groups based on the polishing device used in the second step (nylon bristle brush or rubber prophy cup). The surface examination was carried out with the help of a laser scanner and a contact profilometer. When using full power at a 0 angle, both ultrasonic devices in their research generated rougher surfaces. The wear and roughness values with the greatest wear and roughness values were discovered. When compared to polishing using a nylon bristle brush, polishing with a rubber prophy cup resulted in nearly twice the wear as well as a smoother surface.

MATERIALS AND METHODS

T his study was conducted in The Department of Periodontology of BBDCODS, BBDU, Lucknow and Birbal Sahni Institute of Palaeosciences Research Institute, Lucknow. Patients were selected based upon the following inclusion and exclusion criteria. 45 Periodontally comprised teeth with Grade II and Grade III mobility were extracted.

Inclusion criteria:-

- Patients suffering from periodontal disease.

- Non-carious tooth.

- After RT PCR test or Rapid antigen test for COVID-19, Extraction of Periodontally compromised Miller's Grade II and III mobile teeth.

Exclusion Criteria:-

- Patients who were COVID-19 positive.

- Endodontically treated tooth

- Medically compromised patients

- Pregnant or Lactating women

ARMAMENTARIUM FOR DIAGNOSIS,SCALING AND ROOT PLANING

1. Mouth mirror

2. UNC15 periodontal probe

3. Tweezer

4. Explorer

5. A set of Gracey's curettes (Hu-Friedy, Chicago, IL, USA)

6. Magnifying loupes (3.5x)

7. Extracted teeth

Study Design:

The research was conducted out at the Department of Periodontology, BBDCODS at the University of BBDU, Lucknow.

45 freshly extracted teeth were collected, each tooth was thoroughly rinsed under tap water and was brushed lightly with a soft bristle tooth brush for a minute to remove any blood or food debris and was stored in 0.9 % saline.

The samples collected were randomly assigned to three groups namely,

- Test Group I: -   15 teeth where no SRP was done

- Test Group II : -   15 teeth where SRP was done under naked eye

- Test Group III : -   15 teeth where SRP was done under magnification (3x)

The extracted teeth were mounted on plaster casts and the samples were randomly divided into three groups as mentioned above.In Test Group I, no SRP was done; In test Group II, manual scaling and root planing was performed under naked eyes and In test Group III, manual SRP was done under magnification loupes. Samplesin Groups II and III were instrumented in a single session by a single operator to ensure the same working conditions and pressure applied during scaling. By visual and tactile assessment, the tooth surfaces looked smooth and calculus-free after SRP. Scaling and Root Planing was performed using Gracey curettes (HuFriedy, Chicago, IL, USA) in both groups, and there was no time restriction for SRP.

Methodology:

SEM preparation of a tooth specimen: -

After SRP, all 45 samples were sagittally segmented into less than 12.0 mm x 12.0 mm x 10.0 mm (length, breadth, and height, respectively) using a micromotor device and disc bur. Both control and test group teeth were put in 2.5 percent glutaraldehyde in 0.1 percent M phosphate buffer (pH 7.4) for a day to fix the samples. It was then rinsed and dehydrated using increasing concentrations of ethyl alcohol (70%, 90% and 100%), followed by air drying for two days[19]. The samples were scanned under a scanning electron microscope (SEM) after all of these fixations, dehydration, and air drying procedures.

The SEM study was carried out in Birbal Sahni Institute of Palaeosciences Research Institute, Lucknow.

Scanning Electron Microscope Procedure -:

The samples were completely dried to withstand high vacuum (10^{-5} Pa). The side opposite the site of interest was flattened to enable sample mounting .The samples were mounted on a metallic mounting table which was around 12.0 mm x 12.0 mm in length and width using double sided sticky tape . It was then placed inside sputter coater machine where all the samples were automatically coated with a thin layer of 20 nm to 30 nm of the conductive metal palladium and platinum alloy coating (Pt/Pd) .The idea of coating the specimen is to increase its conductivity in the Scanning Electron Microscope and to prevent the build-up of high voltage charges on the specimen.

After this, the samples were removed from sputter coater machine and scanned under Field Emission Electron Scanning Microscope (FESEM)(JEOL JSM 76610f,

JEOL India Pvt. Ltd.). One at a time each sample was again mounted on another stump holder which was 12.0 mm x12.0 mm in length and width and screwed tightly .The sample was then kept inside a vacuum chamber (as electrons do not travel very far in the air).The lenses of the FESE microscope are replaced by a series of coil- shaped electromagnets through which the electron beam travels. Thus, the image so formed is as a photograph (called an electron micrograph) or as an image on a TV screen.To obtain a broad picture of the surface topography of each specimen, the complete control and test surface of each specimen was scanned first. The scaled area was then examined using SEM, and several indices were determined. Photographs were taken at x50 and x100 magnification for each surface.

The following indices were used to assess the quantity of residual calculus, surface roughness, tooth material loss and presence or absence of smear layer in the specimens:

Remaining Calculus Index (RCI) given by Meyer and Lie in 1977[4]

The amount of remaining calculus was evaluated visually with SEM photographic prints at magnification 72x. Scoring criteria is as follows;

0:      No calculus remaining on the root surface

1:      Small patches of extraneous material, probably consisting of calculus

2:      Definite patches of calculus confined to smaller areas

3:      Considerable amounts of remaining calculus, appearing as one or a few voluminous patches or as several smaller patches scattered on the treated surface.

Loss of Tooth Substance Index (LTSI) given by Meyer and Lie in 1977[4]

The loss of tooth substance was evaluated visually with SEM photographic prints at magnification 72x. Scoring criteria is as follows;

0:    No detectable loss of tooth substance.

1:    Slight loss of tooth substance restricted to localized areas. Most of the cementum is intact.

2:    Definite loss of tooth substance on most of the treated surface, but without deep instrumental marks in the dentin. Cementum may be absent in some areas.

3:    Considerable loss of tooth substance with deep instrumental marks in the dentin. Most of the cementum is removed.

Roughness Loss of Tooth Substance Index (RLTSI) given by Lie and Leknes in 1985[5]

The micro surface roughness on the tooth surface was evaluated visually with SEM photographic prints at magnification 72x. Scoring criteria is as follows;

0:    Smooth and even surfaces or slightly roughened, but without signs of instrumental marks.

1:    Mostly slightly roughened areas with some corrugated regions but no obvious Instrumental marks.

2:    Definitely corrugated areas and some instrumental marks, but also relatively even areas.

3:    Definitely corrugated surface with instrumental scratches over most of the areas.

Presence or absence of Smear Layer.

According to Kawashima and Sato et al. (2007)[6], Scaling and root planing creates a layer of organic and mineralized debris known as smear layer that covers the surface of the instrumented roots and occludes the dentinal tubules. According to the above definition the presence and absence of smear layer was noted.

Thus, the data collected was analyzed for statistical analysis.

Fig. 1: Armamentarium for Scaling and Root Planing.

Fig. 2: Armamentarium for Teeth Specimen Sectioning.

Fig. 3: Specimen Mounted for Sputter Coating with Palladium Platinum Alloy
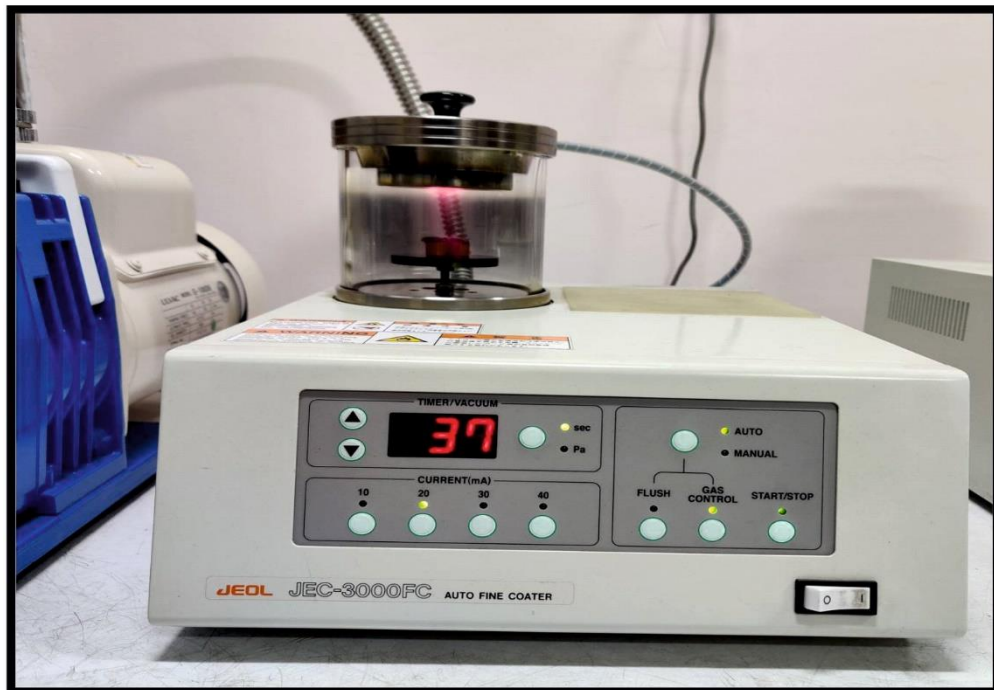
Fig. 4: Sputter Coater Machine.
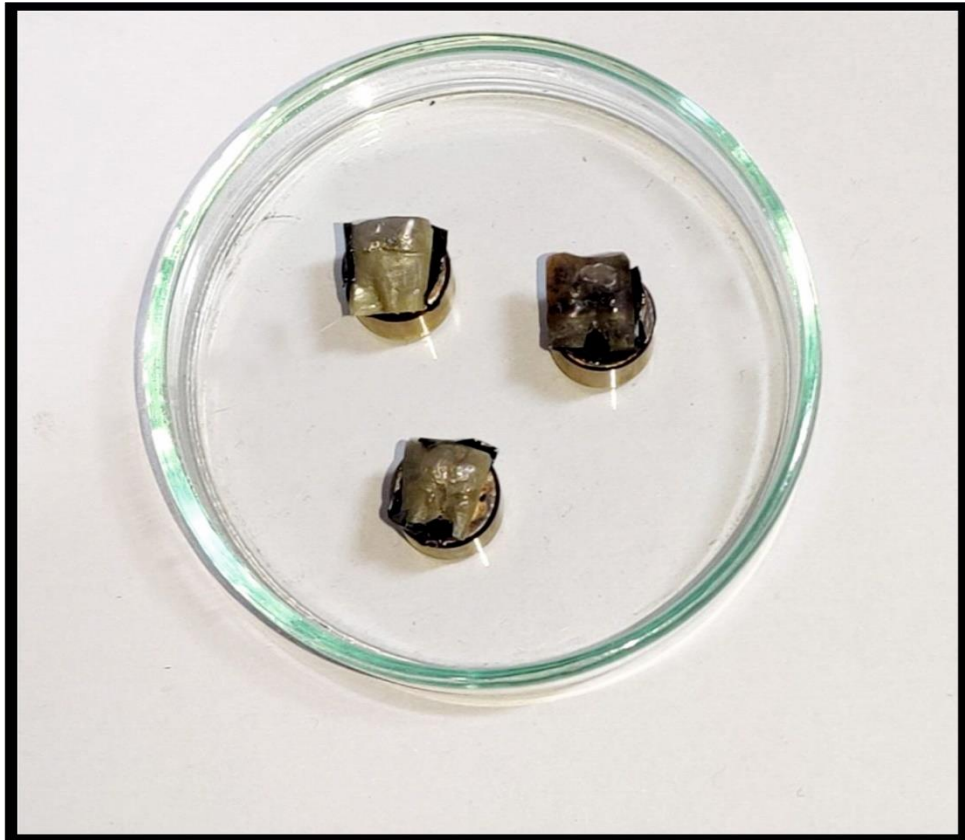
Fig. 5: Specimen Following Palladium-Platinum Coating.

Fig.6: Field Emission Scanning Electron Microscopy.
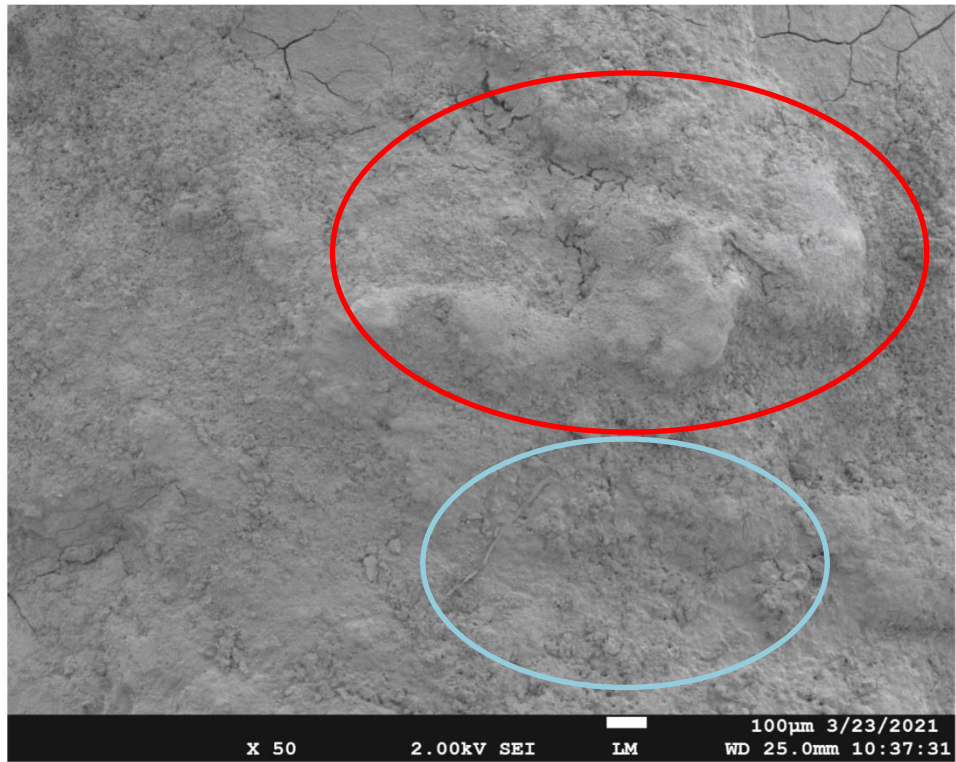
Fig.7: Control Group I: SEM analysis at 50x showing the large mound of calculus (encircled red) and irregular surface due to plaque (blue)
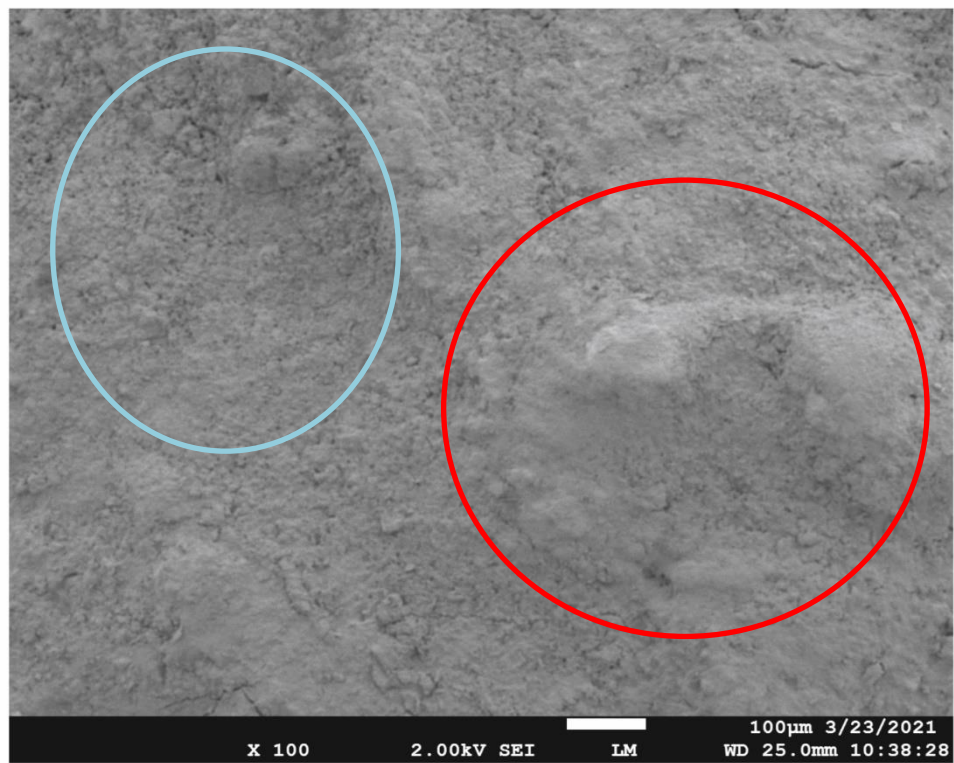
Fig. 8: Control Group I: SEM analysis at 100x. At higher magnification both plaque and calculus are clearly visible.
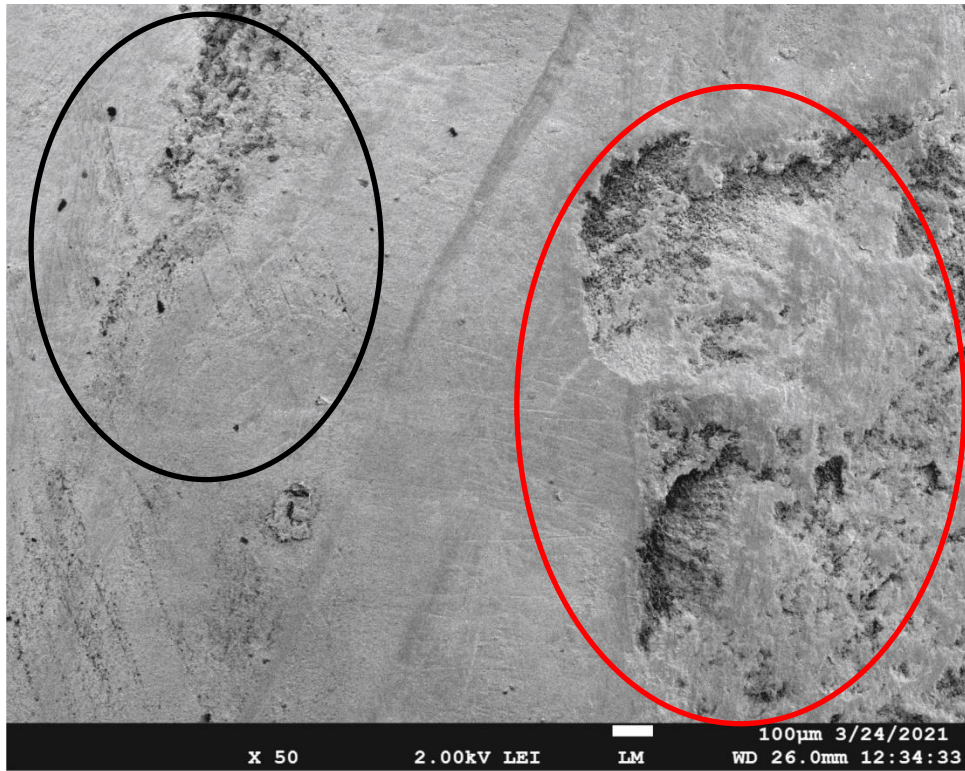
Fig. 9: Test Group II: SEM analysis at 50x showing loss of tooth substance (encircled red); specks of remaining calculus (encircled black).
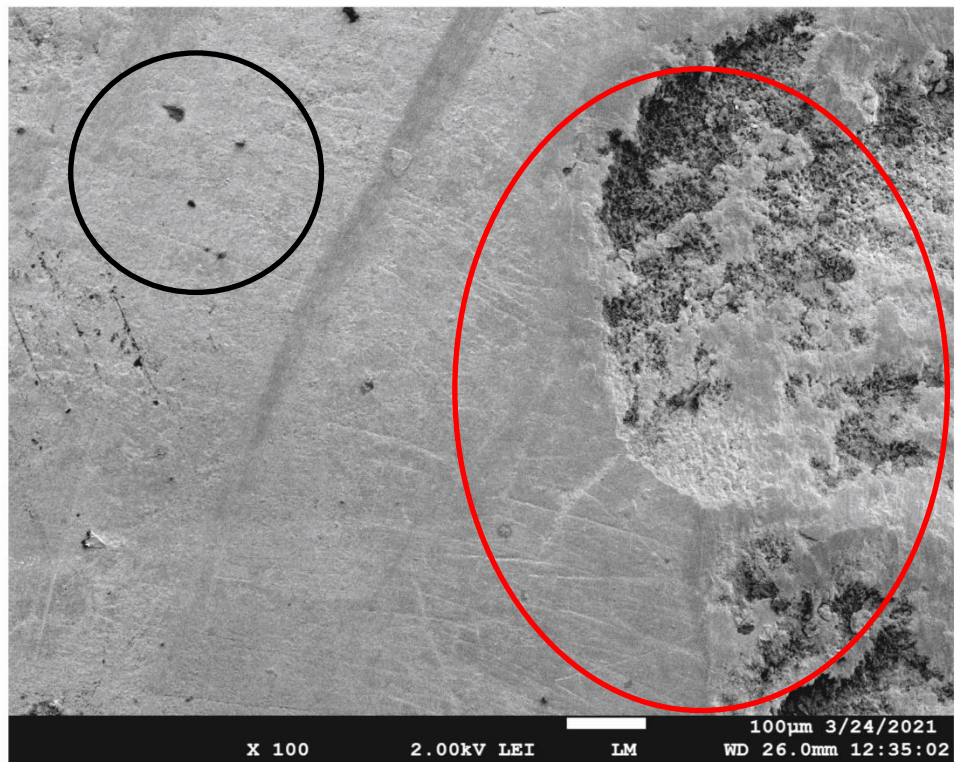
Fig. 10: Test Group-II: SEM analysis at 100x magnification.
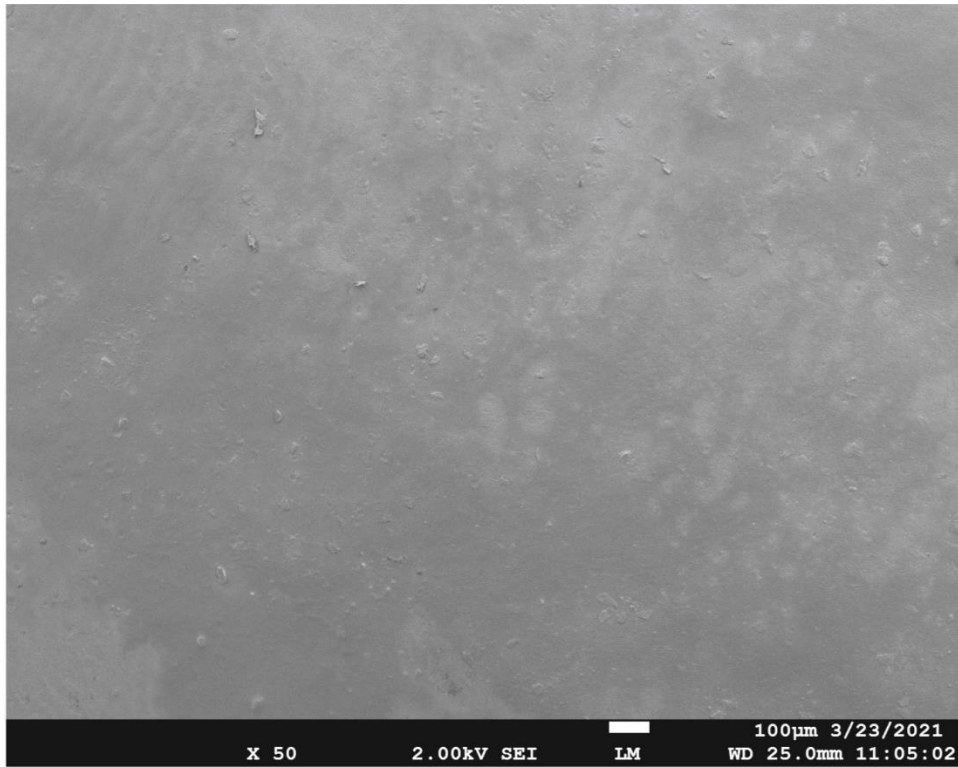
Fig. 11: Test Group III: SEM analysis at 50x showing smoother tooth surface that interprets to no remaining calculus and no loss of tooth
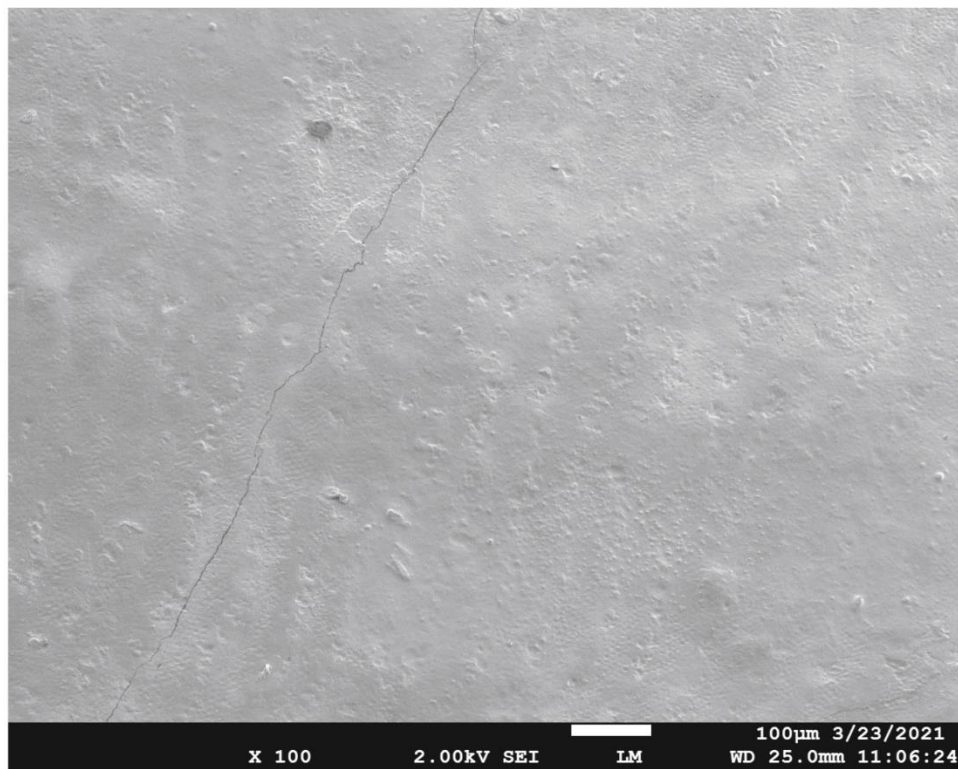
Fig. 12: Test Group-III: SEM analysis at 100x magnification.

RESULTS AND OBSERVATIONS

The study was carried out in Department of Periodontology, BBCDOS, Lucknow and second part of the study was carried out at BirbalSahni Institute of Palaeosciences Research Institute, Lucknow. The aim of the study was to compare SRP done under naked eye to SRP done under magnification evaluated by SEM findings. The objective of the study was to assess and compare LTSI, RLTSI, Remaining Calculus and presence or absence of smear layer.

Table 1: Mean description of Loss of Tooth Substance Teeth Index (LTSI)

Group	Mean	SD
Group I (No SRP done)	2.84	0.48
Group II (SRP done under Naked Eyes)	2.80	0.41
Group III (SRP done under Magnification)	1.13	0.35

The present study comprised of three Groups i.e. Group I (No SRP done), Group II (SRP done under naked eye) and Group III (SRP done under magnification). Table 1, Graph 1 shows the mean description of loss of tooth surface teeth index (LTSI). Mean LTSI among Group I was 2.84±0.48, Group II was 2.80±0.41 and Group III was 1.13±0.35. Group I was reference group in which SRP was not performed. Group I showed more amount of LTSI, since as per SEM studies there was more undulated surfaces due to presence of calculus. So it appeared to have tooth loss when comparison was made presence of calculus. Group II showed more amount of LTSI due to more

instrumentation. Group III showed smoother surface since instrumentation was not aggressive thereby preserving the tooth structure.
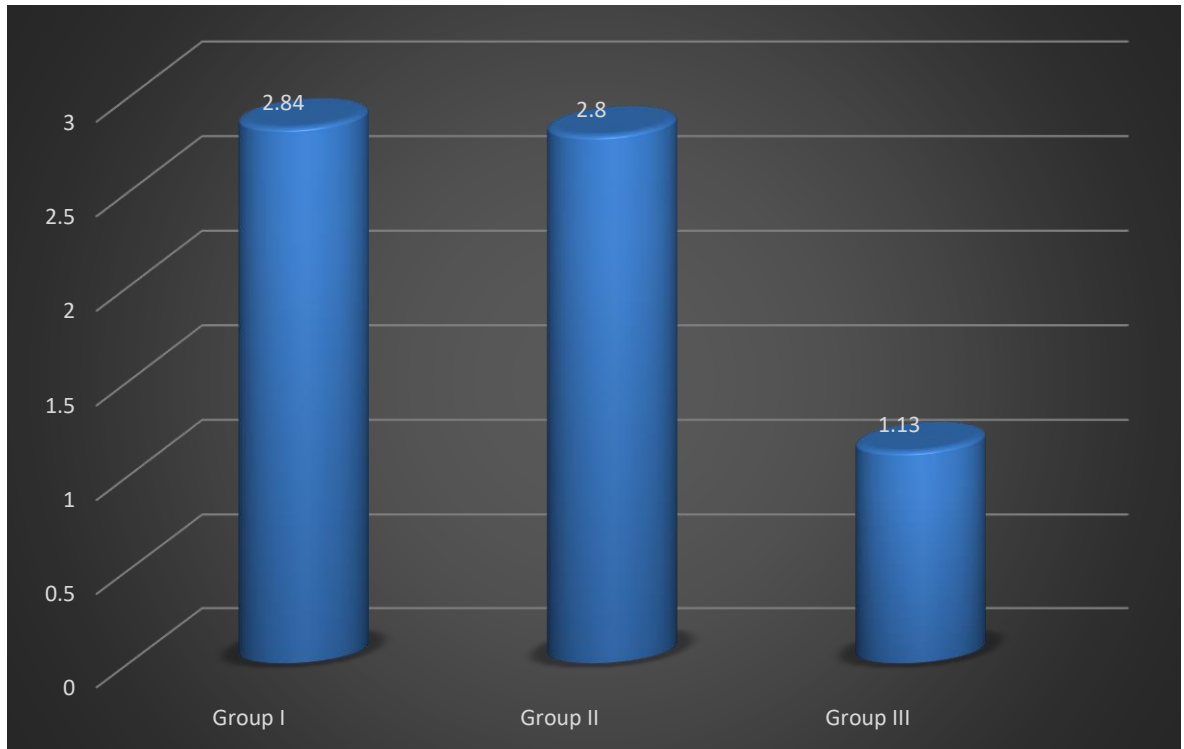
Graph 1: Mean description of Loss of Tooth Surface Teeth Index (LTSI)
Least amount of LTSI is seen in Group III.

Table 2: Intergroup comparison of mean LTSI

Group	t test	p value
Group I vs II	0.58	0.78
Group I vs III	8.59	<0.01**
Group II vs III	8.03	<0.01**

*: statistically significant, **: highly significant

Table 2 shows the intergroup comparison of mean LTSI among the study Groups. Statistically significant difference was found when Group II and Group III were compared to each other ($p<0.05$). Signifying the fact that there was more amount of tooth loss in Group II as compared to Group III.

Table 3: Mean description of remaining calculus index

Group	Mean	SD
Group I	2.87	0.43
Group II	3	0.07
Group III	1.80	0.38

Table 3, graph 2 shows the mean description of remaining calculus index. Mean remaining calculus index among Group I was 2.87±0.43, Group II was 3±0.07 and Group III was 1.80±0.38. As per the results obtained Group II showed more amount of remaining calculus as compared to Group III.

Graph 2: Mean description of remaining calculus index

Table 4: Intergroup comparison of mean remaining calculus index

Group	t test	p value
Group I vs II	0.92	0.47
Group I vs III	9.32	<0.01**
Group II vs III	10.07	<0.01**

*: statistically significant, **: highly significant

Table 4 shows the intergroup comparison of mean remaining calculus index among the study Groups. Statistically significant difference was found when Group III was compared to Group I as well as Group II in respect to remaining calculus index as $p<0.05$.

Table 5: Mean description of root surface roughness index (RLTSI)

Group	Mean	SD
Group I	2.39	0.32
Group II	2.33	0.49
Group III	1.20	0.42

Table 5, graph 3 shows the mean description of root surface roughness index (RLTSI). Mean RLTSI among Group I was 2.39±0.32, Group II was 2.33±0.49 and Group III was 1.20±0.42. Group I showed maximum roughness since SRP was not done. Group II showed more surface roughness as compared to Group III.

Graph 3: Mean description of root surface roughness index (RLTSI)

Table 6: Intergroup comparison of mean root surface roughness index (RLTSI)

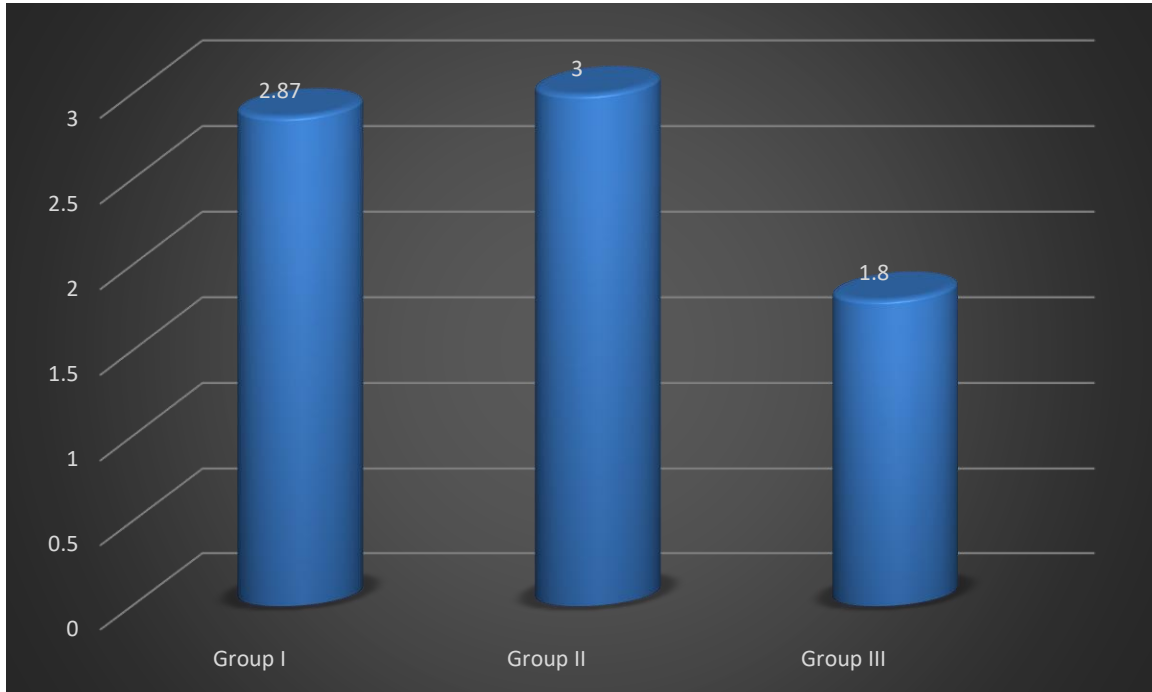| Group	t test	p value
Group I vs II	0.41	0.58
Group I vs III	9.02	<0.01**
Group II vs III	8.67	<0.01**7

*: statistically significant, **: highly significant

Table 6 shows the intergroup comparison of mean RLTSI among the study Groups. Statistically significant difference was found when Group III (SRP done under magnification) was compared Group I (No SRP done) as well as Group II (SRP done under naked eyes) with respect to RLTSI as p<0.05.

Table 7: Comparison of smear layer among the study Groups

Group	Present		Absent	
	N	**%**	**N**	**%**
Group I	15	100	0	0
Group II	9	60	6	40
Group III	1	6.67	14	93.33

Table 7, graph 4 shows the comparison of smear layer among the study Groups. Smear layer was reported among 100%, 60% and 6.67% of the subjects in Group I, II and III respectively.

Graph 4: Comparison of smear layer among the study Groups

Table 8: Intergroup comparison of smear layer

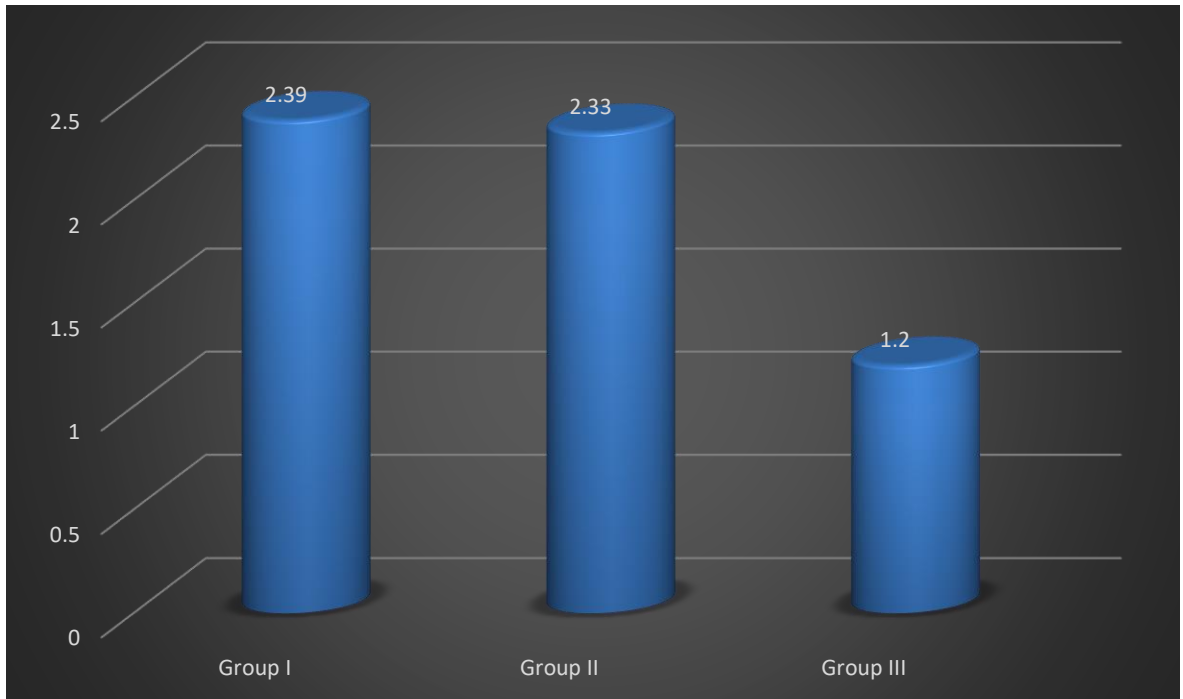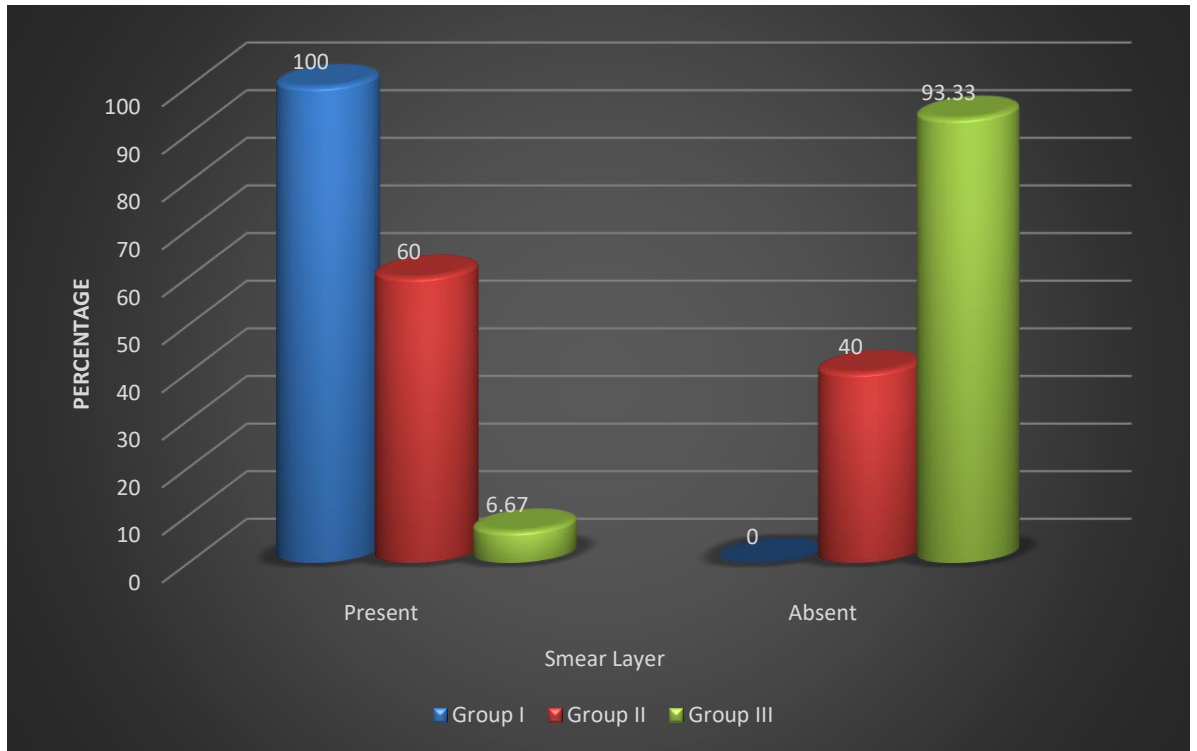| Group	Chi Square test	p value
Group I vs II	5.13	0.009*
Group I vs III	8.47	<0.01**
Group II vs III	4.66	0.03*

*: statistically significant, **: highly significant

Table 8,shows the intergroup comparison of smear layer among the study Groups using chi square test. The results suggest that Group III (SRP done under magnification) showed better results than Group II (SRP done under naked eyes) and control Group I (No SRP done).

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by individual bacteria or Groups of microorganisms that exist within microbial plaque leading to gradual loss of periodontal structures[28].Mechanical disruption and removal of the microbial plaque community is the most reliable method of disrupting the microbial plaque population and Nonsurgical periodontal therapy (NSPT) is the first in a series of treatments used to treat periodontitis. SRP, local drug delivery, antimicrobial treatment are all included in NSPT. Both hand instruments and ultrasonic instruments are used and these drastically reduces the numbers of subgingival microorganisms. The goal of NSPT is to change or eliminate the microbial etiology of gingival and periodontal disease as much as possible, as well as re-establish a biologically acceptable root surface. To attain this periodontal health, plaque, calculus, and some amount of altered cementum contaminated with bacterial products and components (e.g. endotoxin) needs to be removed by thorough scaling and root planing.

According to a series of studies done, it was shown that surface irregularities are known to grow bacterial colonization [29]and plaque formation which further leads to attachment and retention of dental calculus, according to Selvig et al. 1970[30]. Quirynen et al. studied that roughness increases wetness, which is directly linked to plaque development [31]. Healthy root surfaces are characterized by absence or minimally present endotoxins that remain compatible with health. Bacterial Lipopolysaccharides (LPS and Endotoxins) has been identified as cytotoxic and pathogenic factors by research done by Hatfield & Baumhammers[32].

The inflammatory effects of LPS include their ability to stimulate macrophages, activate complement, and lead to bone resorption to mention a few. Due to the presence of these inflammatory factors, the authors showed that the presence of LPS leads to irreversible morphologic changes on periodontally diseased root surfaces[33]. This suggested that the presence of these endotoxins was capable of impairing cell attachment. Aleo et al. found that periodontally affected root surfaces contained an endotoxin-like substance capable of inhibiting tissue culture fibroblast cell proliferation[34].In another in vitro study, they discovered that human gingival fibroblasts did not adhere to a root surface infected with LPS/Endotoxins[35].Due to the presence of bacterial endotoxins, fibroblasts no longer attach and spread on diseased root surfaces, nor does do novel attachment shape form on them, according to studies[36].

The objective for the SRP method, according to Ramford et al. in 1980, was to create a biologically acceptable root surfaces by eliminating all adherent mineralized or non-mineralized bacterial plaque as well as cementum that would contain endotoxins[7].To be effective, Aleo et al suggested that periodontal therapy must either eliminate toxic materials from the root surface of the affected cementum or remove the cementum.As a result of this study, many people believe that endotoxins are found on the cementum[33].Hence, therefore removal of cementum during periodontal treatment may be required. Kathiblou and Ghodssi in 1983 studied that the removal of diseased tissue and the prevention of re-infection are the most important aims for facilitating periodontal repair, with the roughness of the surface being clinically unimportant. The optimal root surface roughness or smoothness for promoting healing and preventing bacterial adherence remains yet to be determined[34].

Nakib et al. (1982)[39], Nyman et al. (1986)[41] and Cadosch et al. (2013)[9]reported that plaque, calculus, and endotoxins are exclusively located on the surface of the root and not within the root surface; though there is a probability of micro-organisms harboring in cemental lacunae also. Kawashima H et al. in 2007, examined that endotoxins loosely attaches on the superficial layer on root surface[6].So, in order to remove the bacterial endotoxins deposits from the tooth surface need to be removed but additional or overzealous SRP is not necessary to lower the endotoxins level at the cost of dentin hypersensitivity. Curetted cementum, according to Aspriello et al. (2009), is made up of newly formed collagen fibrils produced by healthy, functioning fibroblasts associated to the instrumented surface, and it organises itself unambiguously toward the hand curetted root surface[41].

A roughened yet debrided root surface is required for new adhesion. The studies have emphasized the need of a mineralized microscopic rough cementum layer for starting cementogenesis throughout the healing process, but a very rough residual root surface following instrumentation may clearly provide a danger of periodontal pathogen colonisation[42].

Overzealous and repeated SRP would result in unnecessary excessive exposure of root substance exposing the patient to root hypersensitivity, fracture, and pulpitis. Scaling leads to a reduced concentration of endotoxins, which is linked with a healthy periodontium. According to the study done by Fukazawa and Nishimura (1994), curettage of the Superficial Cementum appears to have created a better root surface for new fibrous attachment and the deeper cementum layer is a suitable place for new fibrous connective tissue to adhere to periodontally treated teeth and therefore curettage of this layer should be avoided[7].Smoother root surfaces indicate less build-up of supra

and subgingival plaque, less bacterial adherence, and a lesser degree of periodontal inflammation than rougher and uneven root surfaces[42].

Under naked eye, complete removal of deposits from the tooth surface, particularly inaccessible regions, is relatively challenging. Magnifying the area of interest is the most effective technique to get better vision. Magnification improves the operator's visual acuity, illumination, and intensity, resulting in improved clinical outcomes. Better illumination combined with magnification, According to Worschech CC et al., offers a visible difference in working conditions in diagnostic, prophylactic and surgical modalities. Magnification tools, According to Tascheiri S et al., are beneficial to both clinicians and patients in terms of ergonomics, precision, detailing, accuracy, eyesight, and treatment time[3].It works by reducing the depth of field to the point where just a tiny object is sharply in focus, while everything else blurs out. Hence, Magnifying loupes were used to accomplish all the above in our study.

Scanning Electron Microscopy (SEM) is a popular method for studying and gaining information on the microstructure, morphology and composition of materials such as teeth, composites and ceramics. It has proven to be helpful to in dental research also. Images at high magnification 50x to 10000x and higher can be seen with SEM[43].

The effectiveness of scaling and root planning procedures under magnification is removing deposits from the root surfaces has been clearly demonstrated by this study. Group I served as guideline to assess the normal microstructure of teeth. It was seen in Group II (SRP under naked eye) that some amount of residual calculus was left behind on scaled root surfaces especially in inaccessible areas like interdental and furcation areas. In Group III (SRP under magnification) it was observed that residual calculus

was not present due to the magnified view of the area to be scaled, the operator was able to have controlled precise instrumentation strokes. As previously mentioned, studies have proven that endotoxins are present only in the superficial and not in deeper layers of cementum, thereby the need for repeated instrumentation strokes was eliminated. As a result of which there was less scratches on the teeth surfaces. This is evident when we compare the Remaining Calculus Index in Group II and Group III, we found that Group III showed lesser remaining calculus as compared to Group II which is in accordance with the findings of Meyer and Lie in 1977[4]. Another index was to compare the Loss of Tooth Substance in Group II and Group III, it was seen that Group III showed lesser loss of tooth substance since the number of strokes performed under magnification was lesser. This is also seen with the findings of Meyer and Lie in 1977[4]. When Root Surfaces Roughness was compared, Group II had marginally more roughness as compared to Group III, also because the instrumentation done under magnification in the latter. This is also seen with the findings of Meyer and Lie in 1977[5]. On comparing the smear layer, Group II exhibited the presence of smear layer, whereas Group III had extremely low levels of the same. Presence of smear layer is an indicator of excessive instrumentation, which is also seen in the findings of Kawashima and Sato et al. in 2007[6]. Overall Group III exhibited smoother surface with fewer instrumentation marks, no calculus and minimally present smear layer.

Hence, it can be concluded from the above findings of SEM study that SRP performed with the help of magnifying loupes is far more beneficial in terms of effective calculus removal and preserving the tooth structure.

F rom the present study we can conclude that SRP when done under magnification with the help of magnifying loupes noticeably increased the efficacy of the procedure. This was evident by complete removal of even tiny flecks of calculus which otherwise, might at times be left behind and further become a nidus of plaque accumulation. With more precise strokes, the calculus removal becomes easier. Since, more precise and less number of strokes are carried out, there was lesser instrument marks or scratches on the teeth; lesser amount of overall loss of tooth structure as was supported by SEM study. So, the objective of the study was to assess Roughness, Remaining Calculus and Loss of tooth structure under magnification, with manual SRP. It is possible to assume that magnification proves to be better, precise, controlled, less invasive or destructive modality when compared to carrying out the procedures under naked eye. As much as possible the dental operator must use the magnifying loupes in their clinical practices to enhance both operator and patient treatment outcomes. Further studies need to be done to understand quantity and quality of strokes so as to prevent overzealous instrumentation. Clinically and histologically, SRP under magnification with magnifying loupes leads topositive outcomes.

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BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES (FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"Scanning Electron Microscope (SEM) Study To Compare Mannual Scaling and Root Planing with and Without Magnification-: An In-Vitro Study."** submitted by **Dr Neha Chand** Post graduate student from the **Department of Periodontology** as part of MDS Curriculum for the academic year 2018-2021 with the accompanying proforma was reviewed by the Institutional Research Committee present on **04th January, 2021** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

ANNEXURE – II

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VIIth Institutional Ethics Sub-Committee

IEC Code: 32 (Revised)   **BBDCODS/01/2021**

Title of the Project: Scanning Electron Microscope (SEM) Study To Compare Manual Scaling and Root Planing with and without Magnification-: An In-Vitro Study.

Principal Investigator: Dr. Neha Chand   **Department:** Periodontology

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: Revised, MDS Project Protocol

Dear Dr. Neha Chand,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 07th January 2021.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Sumalatha M.N. Member	Reader, Department of Oral Medicine & Radiology, BBDCODS, Lucknow
4.	Dr. Akanksha Bhatt Member	Reader, Department of Conservative Dentistry & Endodontics, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala
07/01/21

(Dr. Lakshmi Bala)
Member-Secretary
IEC   Member-Secretary
Institutional Ethic Committee.
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. B. Rajkumar)
Principal
PRINCIPAL BBDCODS
Babu Banarasi Das College of Dental Sciences.
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226028

ANNEXURE - III

दूरभाष/Telephone : 0522 - 2742903, 2742902
फैक्स/Fax : 91-0522-2740485, 2740098
ई—मेल/E-mail : registrar@bsip.res.in
वेबसाइट/Website : www.bsip.res.in / www.bsip.india.org

बीरबल साहनी पुराविज्ञान संस्थान
BIRBAL SAHNI INSTITUTE OF PALAEOSCIENCES

1946

53, विश्वविद्यालय मार्ग,
53 University Road,
लखनऊ-226 007
Lucknow-226007
(भारत) (INDIA)

(भारत सरकार के विज्ञान एवं प्रौद्योगिकी विभाग का एक स्वायत्तशासी संस्थान)
(AN AUTONOMOUS INSTITUTE UNDER DEPARTMENT OF SCIENCE & TECHNOLOGY, GOVERNMENT OF INDIA)

संख्या
No. ....
BSIP/SA/(SEM)/2020-21/ C- 1696

Speed Post

दिनांक
Dated ....
06.01.2021

Dr. Vandana A. Pant
Prof. and Head
Department of Periodontology
Babu Banarasi Das College of Dental Sciences
BBD City, Faizabad Road
Lucknow - 227105

विषय / Subject : क्रमवीक्षण इलेक्ट्रॉन सूक्ष्मदर्शी सुविधा का उपयोग / Utilization of SEM Facility in BSIP

महोदय / महोदया / Dear Sir/Madam,

With reference to your email dated Nil addressed to the Director, BSIP, requesting therein to provide SEM Facility. I am directed to inform you that the above facilities can be provided to Dr. Neha Chand, PG 3rd Year Student **on payment basis** on the following rates:

संस्था/सवेतन शोध अध्येता/प्रायोजित परियोजना/चिकित्सा विश्वविद्यालय/**Paid Research Scholars of Universities/ PG**
Colleges:
a) प्रक्रमण एवं स्थापना/Sample Processing and mounting — Rs. 250 per sample
b) स्वर्ण पैलेडियम परतबंदी/Gold Palladium coating — Rs. 300 per stub
c) नमूना परीक्षण एवं फोटोग्राफी/Sample examination and photography — Rs. 300 per exposure

*विश्वविद्यालय/स्नातकोत्तर विद्यालय के अदत्त शोध अध्येता/****Unpaid Research Scholars of Universities/ PG**
Colleges:
a) प्रक्रमण एवं स्थापना/Sample Processing and mounting — Rs. 250 per sample
b) स्वर्ण पैलेडियम परतबंदी/Gold Palladium coating — Rs. 150 per stub
c) नमूना परीक्षण एवं फोटोग्राफी/Sample examination and photography — Rs. 100 per exposure

एकल स्पेक्ट्रमी के ई डी ए एक्स प्रभार/**EDAX charges for single spectrum** — Rs. 1000 per spectrum
क्रांतिक बिंदु सुखाना/**CPD (Critical point drying)** — Rs. 500 per sample
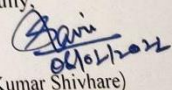
*एकल अदत्त विद्यार्थियों के रेट संबधित विभागाध्यक्ष का प्रमाण—पत्र प्रस्तुत करने पर ही लागू होंगे / **The rates of Unpaid Research Scholar will be applicable subject to production of Certificate from the concerned Head of the Department.
नोट :– भारत सरकार के समय–समय पर प्रतिमानक (Norms) के अनुसार जी.एस.टी @18% देय होंगे / Note: GST @18% will be charged extra as per the Government of India Norms from time to time.

परिचय पत्र के दिखाने पर ही सुविधा का उपयोग दिनांक 22, जनवरी 2021 को किया जा सकता है./ She may utilize the SEM Facility on January 22, 2021. GST NO. 09AAATB6882H2ZF

She may bring one CD-R of 700 MB for Loading images.

भवदीय / Yours faithfully,

(संदीप कुमार शिवहरे / Sandeep Kumar Shivhare)
रजिस्ट्रार /Registrar

आंतरिक प्रतिलिपि/**Copy to:** संयोजक, इलेक्ट्रॉन सूक्ष्मदर्शी समिति / Convener, Electron Microscopy Committee, BSIP, Lucknow the time to be allotted keeping in view Institute requirements

Received

ANNEXURE - IV

Babu Banarasi Das College of Dental Sciences
(A constituent institution of Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

Participant Information Document (PID)

1. **Study title**

   Scanning Electron Microscope (SEM) Study to Compare Manual Scaling and Root Planing with and without Magnification - : An in-vitro Study.

2. **Invitation paragraph**

   You are being invited to take part in a research study, it is therefore important for you to understand why the study is being done and what it will involve. Please take time to read the following information carefully. Ask us for any clarifications or further information. Whether or not you wish to take part is your decision.

3. **What is the purpose of the study?**

   To compare scaling and root planing done under magnification to scaling and root planing done under naked eye assessed by Scanning electron microscope (SEM) findings.

4. **Why have I been chosen?**

   You have been chosen for this study as you are fulfilling the required criteria for this study.

5. **Do I have to take part?**

   Your participation in the research is entirely voluntary. If you do, you will be given this information sheet to keep and will be asked to sign a consent form. During the study you still are free to withdraw at any time and without giving a reason.

6. **What will happen to me if I take part?**

   If you are willing ,you will be one of the patient's whose periodontally compromised teeth Grade II or Grade III mobile teeth will be extracted and will be used in the study.

7. **What do I have to do?**

You do not have to change your regular lifestyles for the investigation of the study.

8. What is the procedure that is being tested?

1. To assess and compare the remaining calculus on the tooth surface by Remaining Calculus Index (RCI) given by Meyer and Lie in 1977

2. To assess loss of tooth substance by Loss of Tooth Substance Index (LTSI) given by Meyer and Lie in 1977

3. To assess roughness loss of tooth substance by Roughness Loss of Tooth Index (RLTSI) given by Meyer and Lie in 1977

4. To assess presence or absence of smear layer under SEM

9. What are the interventions for the study?

Patient with periodontally compromised Grade II and Grade III mobile teeth will be extracted. However you will not have any side effect on your health.

10. What are the side effects of taking part?

There are no side effects on patients of this study.

11. What are the possible disadvantages and risks of taking part?

There are no risks or disadvantages of taking part in this study.

12. What are the possible benefits of taking part?

Periodontally compromised Grade II and III teeth will be extracted containing any pathology. After extraction the pathology will subside and patient can wear removable or fixed prosthesis or implant .Hence, after extraction he/ she will free from any pathology in oral cavity and will boost patients self confidence. Special attention will be given for uneventful healing of the extracted sites.

13. What if new information becomes available?

If additional information becomes available during the course of the research you will be told about these and you are free to discuss it with your researcher, your researcher will tell you whether you want to continue in the study. If you decide to withdraw, your researcher will make arrangements for your withdrawal. If you decide to continue in the study, you may be asked to sign an updated consent form.

14. **What happens when the research study stops?**

If the study stops/finishes before the stipulated time, this will be explained to the patient/volunteer.

15. **What if something goes wrong?**

If any severe adverse event occurs, or something goes wrong during the study, the complaints will be handled by reporting to the institution (s), and Institutional ethical community.

16. **Will my taking part in this study be kept confidential?**

Yes it will be kept confidential.

17. **What will happen to the results of the research study?**

The results of the study will be to compare scaling and root planing done under magnification to scaling and root planing done under naked eye assessed by Scanning electron microscope (SEM) findings on freshly extracted human periodontally compromised Grade II and III mobile teeth. Your identity will be kept confidential in case of any report/publications.

18. **Who is organizing the research?**

This research study is organized by the academic institution (BBDCODS) and Birbal Sahni Institute of Palaeosciences Research Institute, Lucknow.

19. **Will the results of the study be made available after study is over?**

Yes.

20. **Who has reviewed the study?**

The study has been reviewed and approved by the Head of the Dept, and the IEC/IRC of the institution.

21. **Contact for further information**

Dr Neha Chand

Department of Periodontology and Implantology

Babu Banarasi College of Dental Sciences.

Lucknow-227105

Mob- 9555539430

[54]

Dr Vandana A Pant (HOD)

Department of Periodontology and Implantology

Babu Banarasi College of Dental Sciences.

Lucknow-227105

Mob- 9935957775

Dr. Laxmi Bala,

Member Secretary,

Babu Banarasi College of Dental Sciences.

Lucknow

bbdcods.iec@gmail.com

Signature of PI…………………………………………………….

Name……………………………………………………………

Date ………………………………………………………………….

ANNEXURE - V

बाबू बनारसी दास कॉलेज ऑफ डेंटल साइंसेज

(बाबू बनारसी दास वश्ववद्यालय का एक घटक संस्थान)

बीबीडी सटी, फैजाबाद रोड, लखनऊ - 227105 (भारत)

प्रतिभागी सूचना दस्तावेज (पीआईईडी)

1- अध्ययन शीर्षक

स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोप (एसईएम) स्टडी मैनुअल स्टर्लिंग और रूट प्लानिंग की तुलना मैग्रेफिकेशन के साथ और उसके बिना की जाती है -: इन-विट्रो स्टडी।

2- आमंत्रण अनुच्छेद?

आपको एक शोध अध्ययन में भाग लेने के लए आमंत्रित कया जा रहा है, इसलए यह समझना समझना आपके लए महत्वपूर्ण है क अध्ययन क्यों कया जा रहा है और इसमें क्या शामल होगा। कृपया निम्नलखत जानकारी को ध्यान से पढने के लए समय दें। कसी भी स्पष्टीकरण स्पष्टीकरण या आगे की जानकारी के लए हमसे पूछें। चाहे आप भाग लेना चाहते हैं या नहीं, आपका निर्णय है।

3- अध्ययन का उद्देश्य क्या है?

स्केलिंग और रूट प्लानिंग की तुलना मैग्रेफिकेशन के तहत की गई स्केलिंग और रूट प्लानिंग के लिए की जाती है, जो स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोप (एसईएम) निष्कर्षों द्वारा मूल्यांकन की जाती है।

4- मुझे क्यों चुना गया है?

इस अध्ययन के लए आपको चुना गया है क्योंक आप इस अध्ययन के लए आवश्यक मानदंडों मानदंडों को पूरा कर रहे हैं।

5- क्या मुझे भाग लेना है?

शोध में आपकी भागीदारी पूरी तरह से स्वैच्छिक है। यदि आप करते हैं, तो आपको यह जानकारी पत्र दिया

जाएगा और सहमति फॉर्म पर हस्ताक्षर करने के लए कहा जाएगा। अध्ययन के दौरान आप अभी भी कसी भी समय बिना कसी कारण के वापस लेने के लए स्वतंत्र हैं।

6- अगर मैं भाग लेता हूं तो मेरे साथ क्या होगा?

यदि आप इच्छुक हैं, तो आप उस मरीज में से एक होंगे जिनके समय-समय पर समझौता किए गए दांत ग्रेड II या ग्रेड III मोबाइल दांत निकाले जाएंगे और उनका उपयोग अध्ययन में किया जाएगा।

7- मुझे क्या करना है?

अध्ययन की जांच के लए आपको अपने नियमत जीवन शैली को बदलने की ज़रूरत नहीं है।

8- परीक्षण की जा रही प्रक्रिया क्या है?

1. 1977 में मेयर और लाइ द्वारा दिए गए शेष गणना सूचकांक (RCI) द्वारा दांत की सतह पर शेष पथरी का आकलन और तुलना करना।

2. 1977 में मेयर और लाइ द्वारा दिए गए टूथ सब्स्टेंस इंडेक्स (एलटीएसआई) द्वारा दांत के नुकसान के आकलन के लिए

3. 1985 में लाई एंड लेवनेस द्वारा दिए गए टूथ इंडेक्स (RLTSI) की खुरदरापन से दाँत के नुकसान के आकलन के लिए
4. SEM के तहत स्मीयर परत की उपस्थिति या अनुपस्थिति का आकलन करना

9- अध्ययन के लए हस्तक्षेप क्या हैं?

समय-समय पर समझौता किए गए ग्रेड II और ग्रेड III मोबाइल दांतों के साथ रोगी को निकाला जाएगा। हालाँकि आपके स्वास्थ पर इसका कोई दुष्प्रभाव नहीं होगा।

10- भाग लेने के दुष्प्रभाव क्या हैं?

इस अध्ययन के कोई दुष्प्रभाव नहीं हैं।

11- भाग लेने के संभावत नुकसान और जोखम क्या हैं?

इस अध्ययन में कोई जोखम शामल नहीं है।

12- भाग लेने के संभावत लाभ क्या हैं?

समय-समय पर समझौता किए गए ग्रेड II और III दांतों को किसी भी विकृति युक्त निकाला जाएगा। निष्कर्षण के बाद विकृति कम हो जाएगी और रोगी हटाने योग्य या निश्चित कृत्रिम अंग या प्रत्यारोपण पहन सकता है। बाहर निकलने के बाद, वह मौखिक गुहा में किसी भी विकृति से मुक्त हो जाएगा और रोगियों को आत्मविश्वास बढ़ाएगा। निकाले गए स्थलों की असमान चिकित्सा के लिए विशेष ध्यान दिया जाएगा।

13- क्या होगा अगर नई जानकारी उपलब्ध हो जाए?

यदि शोध के दौरान अतिरिक्त जानकारी उपलब्ध हो जाती है तो आपको इनके बारे में बताया जाएगा और आप अपने शोधकर्ता के साथ चर्चा करने के लए स्वतंत्र हैं, आपका शोधकर्ता आपको बताएगा क आप अध्ययन में जारी रखना चाहते हैं या नहीं। यदि आप वापस लेने का

निर्णय लेते हैं, तो आपका शोधकर्ता आपके वापसी के लिए व्यवस्था करेगा। यदि आप अध्ययन अध्ययन में जारी रखने का निर्णय लेते हैं, तो आपको एक अद्यतन सहमति फॉर्म पर करने के लए कहा जा सकता है।

14- शोध अध्ययन बंद होने पर क्या होता है?

यदि अध्ययन निर्धारित समय से पहले समाप्त / खत्म हो जाता है, तो यह रोगी / को समझाया जाएगा।

15- क्या होगा अगर कुछ गलत हो जाए?

यदि कोई गंभीर प्रतिकूल घटना होती है, या अध्ययन के दौरान कुछ गलत हो जाता है, तो शकायतों को संस्था (ओं), और संस्थागत नैतिक समुदाय को रिपोर्ट करके संभाला जाएगा।

16- क्या इस अध्ययन में मेरा हिस्सा गोपनीय रखा जाएगा?

हां इसे गोपनीय रखा जाएगा।

17- शोध अध्ययन के नतीजों का क्या होगा?

अध्ययन के परिणामों को स्केलिंग और रूट प्लानिंग के तहत स्केलिंग और रूट प्लानिंग की तुलना नग्न आंखों के तहत की गई स्कैनिंग इलेक्ट्रान माइक्रोस्कोप (एसईएम) के निष्कर्षों से की गई है, जो ताजे निकाले गए मानव आवधिक रूप से छेड़छाड़ किए गए ग्रेड II और तृतीय श्रेणी के दांतों पर आधारित हैं। किसी भी रिपोर्ट / प्रकाशन के मामले में आपकी पहचान गोपनीय रखी जाएगी

18- शोध का आयोजन कौन कर रहा है?

यह शोध अध्ययन अकादमिक संस्थान (BBDCODS) और बीरबल साहनी इंस्टीट्यूट ऑफ पलायोसाइंसेज रिसर्च इंस्टीट्यूट, लखनऊ द्वारा आयोजित किया जाता है।

20- अध्ययन की समीक्षा कसने की है?

इस अध्ययन की समीक्षा वभाग के प्रमुख और संस्थान के आईईसी / आईआरसी द्वारा की गई और अनुमोदित की गई है।

21- अधक जानकारी के लए संपर्क करें

डॉ नेहा चंद
पीरियोडोंटोलॉजी और इम्प्लांटोलॉजी विभाग
बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज।
लखनऊ-227,105
मोब- 9555539430

डॉ वंदना ए पंत (HOD)
पीरियोडोंटोलॉजी और इम्प्लांटोलॉजी विभाग
बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज।
लखनऊ-227,105
मोब- 9935957775

डॉ लक्ष्मी बाला

सदस्य सचव

बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज

लखनऊ

bbdcods.iec@gmail.com

पीआई का हस्ताक्षर ……………………………………

नाम ……………………………………………………………

दिनांक ………………………………

ANNEXURE – VI

Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

Consent Form (English)

Title of the Study ………..

Study Number……..
Subject's Full Name……….
Date of Birth/Age ………
Address of the Subject…………………….
Phone no. and e-mail address………………
Qualification ……………………………….
Occupation: Student / Self Employed / Service / Housewife/
Other (Please tick as appropriate)
Annual income of the Subject………………
Name and of the nominees(s) and his relation to the subject……………. (For the purpose of compensation in case of trial related death).

1. I confirm that I have read and understood the Participant Information Document dated ……..for the above study and have had the opportunity to ask questions. **OR** I have been explained the nature of the study by the Investigator and had the opportunity to ask questions.
2. I understand that my participation in the study is voluntary and given with free will without any duress and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.
3. I understand that the sponsor of the project, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my Identity will not be revealed in any information released to third parties or published.
4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).
5. I permit the use of stored sample (tooth/tissue/blood) for future research. **Yes [ ]   No [ ]**
   Not Applicable [ ]
6. I agree to participate in the above study. I have been explained about the complications and side effects, if any, and have fully understood them. I have also read and understood the participant/volunteer's Information document given to me.

Signature (or Thumb impression) of the Subject/Legally Acceptable
Representative:……………..
Signatory's Name…………….   Date ……….
Signature of the Investigator…………………   Date………..
Study Investigator's Name……………………...   Date………..
Signature of the witness……………………   Date………..
Name of the witness………………………….
Received a signed copy of the PID and duly filled consent form
Signature/thumb impression of the subject or legally   Date……..

ANNEXURE – VII

Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

<u>सहमति पत्र</u>

अध्ययन शीर्षक...

अध्ययन संख्या..

प्रतिभागी के पूर्ण नाम...

जन्म तिथि / आयु..

प्रतिभागी का पता ....

फोन नं. और ई-मेल पता ....

योग्यता ....

व्यवसाय: छात्र / स्व कार्यरत / सेवा / ग्रहिणी ....

अन्य (उचित रुप मे टिक करें) ....

प्रतिभागी की वार्षिक आय ....

प्रत्याशीयो के नाम और प्रतिभागी से संबंध...(परीक्षण से संबंधित मौत के मामले मे मुआवजे के प्रयोजन के लिए)

1. मेरी पुष्टि है कि मैंने अध्ययन हेतु सूचना पत्र दिनांक .... को पढ व समझ लिया तथा मुझे प्रश्न पुछने या मुझे अध्ययन अन्वेषक ने सभी तथ्यों को समझा दिया है तथा मुझे प्रश्न पुछने के समान अवसर प्रदान किए गये।

2. मैंने यहाँ समझ लिया कि अध्ययन में मेरी भागीदारी पूर्णत: स्वैच्छिक है और किसी भी दबाव के बिना स्वतंत्र इच्छा के साथ दिया है किसी भी समय किसी भी कारण के बिना , मेरे इलाज या कानूनी अधिकारो को प्रभावित किए बिना , अध्ययन में भाग न लेने के लिए स्वतंत्र हूँ ।

3. मैंने यह समझ लिया है कि अध्ययन के प्रायोजक , प्रायोजक की तरफ से काम करने वाले लोग, आचार समिति और नियामक अधिकारियों को मेरे स्वास्थ्य रिकार्ड को वर्तमान अध्ययन या आगे के अध्ययन के सन्दर्भ देखने के लिए मेरी अनुमति की जरूरत नही है, चाहे मैने इस अध्ययन से नाम वापस ले लिया है। हॉलाकि मै यह समझता हूँ कि मेरी पहचान को किसी भी तीसरे पक्ष या प्रकाशित माध्यम में नही दी जायेगी।

4. मै इससे सहमत हूँ कि कोई भी डेटा या परिणाम जो इस अध्ययन से प्राप्त होता है उसका वैज्ञानिक उद्देश्य (ओं) के उपयोग के लिए मेरी तरफ से कोई प्रतिबंध नही है।

5. भविष्य के अनुसंधान के लिए भंडारित नमूना (ऊतक/रक्त) पर अध्ययन के लिए अपनी सहमति देता हूँ।

हॉ [   ]   नही [   ]   अनउपयुक्त [   ]

ANNEXURE – VIII

DATA COLLECTION

LOSS OF TOOTH SURFACE TEETH INDEX (LTSI)

Test group I (NO SRP)	Test Group II (SRP done under Naked Eyes)	Test Group III (SRP done under Magnification)
0	3	1
0	3	1
0	3	1
0	3	1
0	3	2
0	3	1
0	3	1
0	2	1
0	2	1
0	3	1
0	2	2
0	3	1
0	3	1
0	3	1
0	3	1

Loss of tooth substance Index by TRYGGVE LIE AND KNUT MEYER in 1977-[4]

0: No detectable loss of tooth substance
1: Slight loss of tooth substance restricted to localized areas. Most of the cementum is intact
2: Definite loss of tooth substance on most of the treated surface, but without deep instrumental marks in the dentin. Cementum may be absent in some areas
3. Considerable loss of tooth substance with deep instrumental marks in the dentin. Most of the cementum is removed.

Remaining Calculus Index

Test group I (NO SRP)	Test Group II (SRP done under Naked Eyes)	Test Group III (SRP done under Magnification)
3	2	1
3	2	1
3	2	0
3	1	1
3	1	1
3	2	1
3	1	0
3	2	0
3	2	1
3	2	1
3	2	1
3	2	1
3	2	1
3	2	1
3	2	1

Remaining calculus Index by TRYGGVE LIE AND KNUT MEYER in 1977-[4]

0: No calculus remaining on the root surface

1: Small patches of extraneous material, probably consisting of calculus

2: Definite patches of calculus confined to smaller areas

3: Considerable amounts of remaining calculus, appearing as one or a few voluminous patches or as several smaller patches scattered on the treated surface.

Root Surface Roughness Index (RLTSI)

Test group I (NO SRP)	Test Group II (SRP done under Naked Eyes)	Test Group III (SRP done under Magnification)
0	3	2
0	3	2
0	2	1
0	2	1
0	3	1
0	3	1
0	3	1
0	2	1
0	2	2
0	2	1
0	2	1
0	2	1
0	2	1
0	2	1
0	2	1

Root surface roughness Index by KNUT MEYER AND TRYGGVE LIE 1977-[5]

0: Smooth and even surfaces or slightly roughened, but without signs of instrumental marks
1: Mostly slightly roughened areas with some corrugated regions but no obvious Instrumental marks
2: Definitely corrugated areas and some instrumental marks, but also relatively even areas
3: Definitely corrugated surface with instrumental scratches over most of the areas

PRESENCE AND ABSENCE OF SMEAR LAYER

Test group I (NO SRP)	Test Group II (SRP done under Naked Eyes)	Test Group III (SRP done under Magnification)
PRESENT	PRESENT	ABSENT
PRESENT	PRESENT	ABSENT
PRESENT	PRESENT	PRESENT
PRESENT	ABSENT	ABSENT
PRESENT	ABSENT	ABSENT
PRESENT	PRESENT	ABSENT
PRESENT	PRESENT	ABSENT
PRESENT	PRESENT	ABSENT
PRESENT	PRESENT	ABSENT
PRESENT	PRESENT	ABSENT
PRESENT	PRESENT	ABSENT
PRESENT	ABSENT	PRESENT
PRESENT	ABSENT	ABSENT
PRESENT	PRESENT	ABSENT
PRESENT	ABSENT	ABSENT

According to Kawashima and Sato et al. (2007)[6], Scaling and root planing creates a layer of organic and mineralized debris known as smear layer that covers the surface of the instrumented roots and occludes the dentinal tubules.

ANNEXURE – IX

STATISTICAL ANALYSIS

Data were tabulated and examined using the Statistical Package for Social Sciences Version 22.0 (IBM SPSS Statistics for Mac, Armonk, NY: IBM Corp, USA). Descriptive statistical analysis had been carried out in the present study. Results on continuous measurements are presented as Mean±SD. Categorical data has been presented as frequency distribution. The statistical power calculation was based on the assumption that the data were normally distributed. P-value of <0.05 was considered as significant. Difference between two groups was determined using chi square test and student T test for categorical data and continuous data respectively.

The statistical analysis for the present study was done by applying the following formulae:

1. **Mean**: The mean (or average) is the most popular and well known measure of central tendency. It can be used with both discrete and continuous data, although its use is most often with continuous data. The mean is equal to the sum of all the values in the data set divided by the number of values in the data set. So, if we have n values in a data set and they have values x_1, x_2, ..., x_n, the sample mean, usually denoted by $\bar{x}$ (pronounced x bar), is:

$$\bar{x} = \frac{(x_1 + x_2 + \cdots + x_n)}{n}$$

This formula is usually written in a slightly different manner using the Greek capitol i.e.:

Sample Mean	Population Mean
$\bar{x} = \dfrac{\Sigma x}{n}$	$\mu = \dfrac{\Sigma x}{N}$

where Σx is sum of all data values

N is number of data items in population

n is number of data items in sample

2. **Standard deviation**: the standard deviation (SD, also represented by the lower case Greek letter sigma σ or the Latin letter s) is a measure that is used to quantify the amount of variation or dispersion of a set of data values. A low standard deviation indicates that the data points tend to be close to the mean (also called the expected value) of the set, while a high standard deviation indicates that the data points are spread out over a wider range of values.

$$\sigma = \sqrt{\frac{\Sigma \, (x - \bar{x})^2}{n}}$$

σ = lower case sigma

Σ = capital sigma

$\bar{x}$ = x bar

3. **Chi-square test**: A chi-squared test, also written as χ^2 test, is any statistical hypothesis test where the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true. The chi-squared test is used to determine whether there is a significant difference between the expected frequencies and the observed frequencies in one or more categories.

4. **t test**: A student *t*-test is any statistical hypothesis test in which the test statistic follows a Student *t*-distribution under the null hypothesis. It can be used to determine if two sets of data are significantly different from each other. It is most commonly applied when the test statistic would follow a normal distribution if the value of a scaling term in the test statistic were known. When the scaling term is unknown and is replaced by an estimate based on the data, the test statistics (under certain conditions) follow a Student's *t* distribution.

ANNEXURE – X

PLAGARISM REPORT

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