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IN VITRO EVALUATION OF THE EFFECTS OF DIFFERENT PERIODONTAL TOOLS AND MECHANICAL INSTRUMENTATION ON TOOTH RESTORATION INTERFACE

DOCTOR OF PHILOSOPHY THESIS

DENİZ FINDIK BALCI, DDS

SUPERVISOR
Prof. Dr. Bahar EREN KURU
Co-SUPERVISOR
Assist. Prof. Dr. Ogül Leman TUNAR

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Owner of the Thesis : Deniz FINDIK BALCI

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This study have approved as a Doctorate Thesis in regard to content and quality by the Jury.

	Title, Name-Surname (Institution)
Chair of the Jury:	Prof. Dr. Ceyda ÖZÇAKIR TOMRUK Yeditepe University
Supervisor:	Prof. Dr. Bahar EREN KURU Yeditepe University
Member/Examiner:	Prof. Dr. Funda YALÇIN İstanbul University
Member/Examiner:	Prof. Dr. Hare GÜRSOY Yeditepe University
Member/Examiner:	Dr. Öğr. Üyesi Süleyman Emre Meşeli İstanbul Aydın University

APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ..... and numbered .....

Prof. Dr. Bayram YILMAZ

Director of Institute of Health Sciences

DECLERATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the awards of any other degree except where due acknowledgement has been made in the text.

11/06/2021

Deniz FINDIK BALCI

DEDICATION

To my mother *Diğdem* who has guided my path into the mesmerizing world of words and made me fall in love with reading,

To my father *Hakan* who has shown me that if one has control over their words, they may have control over their world,

And to the most supportive person I have ever known;
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LIST OF SYMBOLS AND ABBREVIATIONS

DPB	Dental Plaque Biofilm
LOD	Linear Oscillating Device
AP	Air Polisher
APP	Air Polishing Powder
$Nahco_{3:}$	Sodium Bicarbonate
SR	Surface Roughness
$Ca_{10}(PO_4)_6(OH)$	Hydroxyapatite
DEJ	Dentinoenamel Junction
Gpa	Gigapascal
Mpa	Megapascal
LPS	Lipopolysaccharide
Eh	Activity of Electrons
SPC	Supportive Periodontal Care
CAL	Clinical Attachment Level
PPD	Probing Pocket Depth
Bop	Bleeding On Probing
US	Ultrasonic Scaler
VAS	Visual Analog Scale
RANKL	Receptor Activator Of Nuclear Factor Kappa Beta
CFU	Colony Forming Unit
Ra	Roughness Average / The Arithmetic Mean Of The Absolute Values of the Roughness Profile Ordinates
FEP	Fluorethylenepropylene
CA	Cellulose Acetate
GI	Gingival Index
PI	Plaque Index
DS	Dentin Sample
CS	Ceramic Sample
SEM	Scanning Electron Microscope
SD	Standard Deviation

CAD/CAM	Computer Aided Design and Computer Aided Manufacturing
Tin	Titanium Nitrite Coated Curette
AP-S	Supragingival AP With the Supra Nozzle and a Trehalose APP
With	an ≈65µm Grain Size
AP-P	Subgingival AP With The Perio Nozzle and a Trehalose APP
With	an ≈30µm Grain Size
W	Watts
CI	Confidence Interval

ABSTRACT

Fındık Balcı, D. (2021). *In Vitro* Evaluation of The Effects Of Different Periodontal Tools And Mechanical Instrumentation On Tooth Restoration Interface, Yeditepe University, Institute of Health Sciences Department of Periodontology, PhD thesis, İstanbul.

The aim of this thesis was to evaluate the effects of periodontal instrumentation on surface roughness and surface characteristics on an *in vitro* interface model including a tooth, ceramic and resin cement surface. 100 blocks were obtained by cementing 100 dentin specimens made from bovine incisors (4x4x9mm) to the reciprocating pressed ceramic specimens (4x4x9mm) produced by the lost wax technique by using a dual cure resin cement. Two specimens were cemented to form blocks and polished after cementation. Blocks were randomly divided into 5 groups and instrumented with 5 different tools: titanium nitride coated curette (TiN), ultrasonic scaler (US), the linear oscillating device (LOD), an air polisher with the supra nozzle using ≈*65µm* powder (AP-S) and a subgingival nozzle using ≈*30µm* powder (AP-P). At the end of each preparation step and periodontal instrumentation, profilometric analysis was conducted. The profilometric analysis revealed an increase in the surface roughness (Ra) of dentin, ceramic and resin cement surfaces for all the instrumentation tools ($p<0.05$), however on the ceramic surface TiN and AP-P did not increase the Ra significantly. The intra-group mean Ra changes of each surface after instrumentations were also compared between the groups. The tools causing the least change respectively on the dentin, ceramic and resin cement surfaces were: TiN; AP-P; TiN, whereas the tools causing the most change respectively; were US; US; AP-S. Also, an additionally prepared block representing each step of the experiment was evaluated under the scanning electron microscope (SEM). SEM findings were found compatible with profilometric results.

Keywords: periodontal instrumentation, restorative margins, restoration interface

ABSTRACT (Turkish)

Fındık Balcı, D. (2021). Periodontal Tedavide Kullanılan Farklı Alet ve Mekanik Sistemlerin Protetik Restorasyon ve Diş Birleşimi Bölgesinde Yarattığı Etkilerin *In vitro* Olarak İncelenmesi, Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Periodontoloji Anabilim Dalı, Doktora Tezi, İstanbul.

Bu tezin amacı periodontal enstrümantasyonda, tam seramik restorasyonların marjinal bölgesinde bulunan diş, seramik ve rezin siman birleşimi üzerindeki etkilerinin; yüzey pürüzlülüğü ve yüzey özellikleri açısından *in vitro* bir model üzerinde değerlendirilmesidir. Sığır kesici dişlerinden elde edilen 100 dentin örnek (4x4x9mm), bu örneklere karşılık gelecek şekilde "kayıp mum" tekniğiyle hazırlanıp preslenmiş 100 tam seramik örnek (4x4x9mm) ile dual etkili rezin siman kullanılarak simante edilmiş ve bu şekilde hazırlanan bloklar simantasyon sonrasında cilalanmıştır. Bloklar randomize bir şekilde 5 gruba ayrılmış ve her grup aşağıda belirtilen 5 farklı alet/mekanik sistem ile enstrümante edilmiştir: titanyum nitrit kaplı küret (TiN), ultrasonik cihaz (US), doğrusal salınım yapan cihaz (LOD), supra uç ve ≈*65µm* cila tozu ile uygulanan "air polishing" ve subgingival uç ve ≈*30µm* cila tozu ile uygulanan "air polishing". Örneklerin hazırlığındaki her basamaktan ve periodontal enstrümantasyondan sonra profilometrik analiz gerçekleştirilmiştir. Enstrümantasyon sonrası dentin, seramik ve rezin siman yüzeylerde yüzey pürüzlülüğünde (Ra) artış olduğu görülmüştür ($p<0.05$). Buna karşın seramik yüzeyler üzerinde TiN ve AP-P uygulamaları Ra'yı anlamlı derecede etkilememiştir. Her enstrümantasyon yöntemine ait grup-içi ortalama Ra değişimleri gruplar arası karşılaştırıldığında; dentin, seramik ve rezin siman yüzeylerde en az artış sırası ile TiN, AP-P, TiN; en çok artış US, US ve AP-S olmuştur. Ek olarak deneysel hazırlıkların tüm basamaklarını ve enstrümantasyon metodlarını temsilen birer örnek blok hazırlanmış ve bu bloklar taramalı elektron mikroskobu ile değerlendirilmiştir. Sonuçların profilometrik değerlendirme ile uyum içerisinde olduğu gözlemlenmiştir.

Anahtar Kelimeler: periodontal enstrümantasyon, restoratif kenar, restoratif arayüz

1. INTRODUCTION AND PURPOSE

Periodontal diseases are multifactorial, dental plaque biofilm (DPB) associated, chronic, inflammatory diseases of the oral cavity (1). The primary etiological factor and the initiating cause of periodontal diseases is the presence of dysbiotic DPB besides its interaction with the host response (2-4). Gram-negative, anaerobic or microaerophilic bacteria are associated with a dysbiotic DPB (5-7). Once the homeostatic relationship between the host response and DPB is disturbed, disease formation occurs (3,4,8).

The rationale behind mechanical periodontal therapy in the first place is preventing disease formation and/or disease progression by the removal of DPB, calculus and other contributing factors (9,10). Hand instruments and ultrasonic/sonic power-driven instruments are the most commonly used tools for mechanical periodontal therapy.

Hand instruments and power-driven instruments are shown to yield comparable clinical results in the literature (11-20) and are recommended to be used alone or in combination (1). Power-driven instruments are also found to be preferred in general over hand instruments by clinicians due to their ease of use and time efficiency (20).

Recently, novel tools are introduced to the market in order to overcome the limitations and disadvantages (11,21-26) of the conventional tools. A power-driven instrument, the linear oscillating device (LOD)[1] was introduced as an alternative, gentle and effective tool for mechanical periodontal therapy. The tip of LOD oscillates linearly parallel to the root surface (27) during instrumentation. The patient perception of this instrument was shown to be superior to other power-driven instruments (28,29). It was found to cause less hypersensitivity compared to hand instruments (30,31). Also, clinical studies concluded that LOD was effective in removing calculus but slower compared to conventional methods (28,32-34).

[1] **The Vector™Paro System**, Dürr Dental, Bietigheim-Bissingen, Germany

Another method is the use of air polishers (AP). Even though the use of APs is limited to supragingival use in the past, with the introduction of a new disposable nozzle enabling subgingival application at a limited pressure by Moëne et al. (35) in 2010; it has gained a wider use in periodontal mechanical treatment. If used appropriately, APs are shown to provide effective, efficient and safe removal of the subgingival DPB (36-46).

Different air polishing powders (APPs) can be used during air polishing including sodium bicarbonate-based ($NaHCO_3$) powders, aluminum hydroxide, calcium carbonate, bioactive glass, glycine, erythritol and a newly introduced APP on the market as trehalose.

Even though the number of studies is very scarce, the use of trehalose APP is shown to provide comparable outcomes to sonic instruments in a clinical study (40) and has reduced bacterial counts (41). Animal studies has shown that trehalose inhibits inflammatory cytokine production (47) and suppresses osteoclastic differentiation (48).

Full ceramic restorations are used increasingly in dentistry (49) due to the higher esthetic demands of patients, improvements in the mechanical and esthetic properties of the material and the possibility of using minimally invasive preparation techniques (50).

One of the challenges faced today by clinicians is to provide an appropriate periodontal therapy to patients who have fixed ceramic restorations. Clinicians should understand the effect of their choice of instruments on the tooth surface, tooth-restoration interface and the restoration; a site where periodontal instrumentation is necessary to ensure and maintain periodontal health.

A review of the literature reveals a positive relationship between an increase in DPB retention and an increase in surface roughness (SR) (51-63). It has also been shown that periodontal instrumentation plays a role in increasing SR and affects surface characteristics (9).

Restorative margins are considered to be retention areas for DPB accumulation. Since the margin is an interface of three different surfaces including the tooth, the material

used for cementation and the restoration (64-71). Knowing that periodontal therapy carries an utmost importance in providing health around restorations/prostheses, choosing the correct technique/instrument is crucial during periodontal instrumentation.

The aim of this study is to evaluate the effects of different techniques and instruments that are used for mechanical periodontal therapy on SR and surface characteristics on the marginal region of all-ceramic restorations including a tooth, ceramic and resin cement surface.

2. GENERAL INFORMATION

2.1. Surface Characteristics and Physical Structure of the Root

Teeth are unique mineralized structures. The coronal part of an erupted tooth is located inside the oral cavity; a niche very rich in microorganisms. Since the oral cavity is in relation to the external environment and relatively outside the body; the location of the coronal part of the tooth can also be considered as the external environment. Anatomically this structure is named the crown. On the other hand, the radicular parts of an erupted tooth are embedded inside the human body where they are in direct relation with the nerves, blood vessels and bone. This part is called the root. The structures that make up the root from the outermost layer to the innermost layer are sequently the cementum layer, the dentin layer, and the pulpal tissue (Figure 2.1). These anatomical structures may be covered by a layer of debris called the *smear layer* caused by abrasion, dental caries or subgingival instrumentation (72).

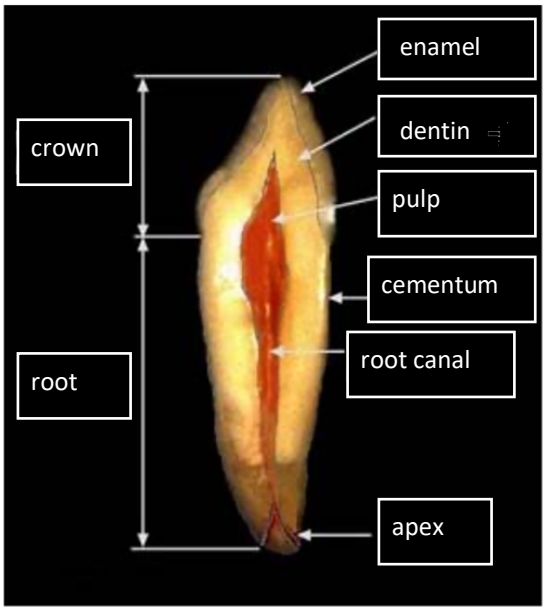


Figure 2.1. Anatomy of human tooth (73)

2.1.1. Dentin

Dentin is a calcified tissue constituting most of the human tooth. It has a mesodermal origin. It forms from the dental papilla of the tooth bud and it circumscribes the pulp. In the anatomic crown, dentin is covered by the enamel layer and in the anatomic root; it is covered by the cementum layer. It has a porous structure. Compared to cementum and bone; dentin has a more mineralized composition, however, compared to enamel; it is less mineralized. As a result, dentin is softer than enamel but harder than bone and cementum. Unlike enamel; dentin formation continues after the eruption of the tooth and continues throughout the life of the pulp (74).

Dentin is a part of the dentin-pulp complex and plays an important role in the continuum of hard and soft tissues (75,76):

Dentin has four functions (74):

1) Formative function: It produces primary and secondary dentin by odontoblasts.
2) Nutritive function: It supplies mineral ions, proteins and water to dentin through the blood supplies of odontoblasts and their processes.
3) Sensory functions: Pain sensation is mediated by the nerve fibers inside the pulp.
4) Defensive/reparative function

Seventy percent of the weight of dentin is hydroxyapatite ($Ca_{10}(PO_4)_6(OH)$) with some other minerals causing impurities (CO_3, Mg, Na, K, Cl) and the remaining %20 of dentin is organic compounds and %10 water (75,76).

The main organic compound present is highly cross-linked type I collagen (%90) however small amounts of type III, type V and type VI collagen are also present. Different kinds of non-collagenous proteins, growth factors, enzymes and lipids can also be found in small amounts (74,76).

Dentin is made by odontoblasts through a process called dentinogenesis. Odontoblasts are accepted as a part of both the pulp and dentin. The bodies of odontoblasts are inside the pulp cavity whereas their cell processes (Tomes fibers) extend into the tubules of mineralized dentin. Due to the presence of these cell processes, dentin is considered to be a living tissue. It reacts to physiologic and pathologic stimuli (74).

Odontoblasts are highly specialized cells with differentiated terminal parts. Even though their main function is dentin formation and maturation, recent studies have shown that they have more diverse functions such as taking part in the innate immune defense of the dentin pulp complex and regulation of pulpal pain (75).

There are dentinal tubules present in the dentin. These canals remain from dentinogenesis. They extend through the entire width of the dentin. In each tubule a cytoplasmic cell process is present. Also, the interior part of each tubule is lined with a layer of peritubular dentin which is much more mineralized than the intertubular dentin. Dental tubules lie in a slight S-curve in the tooth crown but they are straighter in the root areas (74).

Dentinal tubules are filled with the dentinal fluid. Dentinal fluid is a transudate of plasma that contains the components required for mineralization (water, matrix proteins, matrix modifying proteins, mineral ions) (74).

Both the composition and the structure of dentin show differences in different parts of the tooth (75). In the dentinoenamel junction (DEJ) and dentinocemental junction the surface area of the dentin is much greater compared to the surface area of dentin facing the pulp cavity. Dentin layer is made from outside towards inside by odontoblast. At the side facing the pulp cavity odontoblasts are forced closer together. As a result, the number of tubules per mm^2 increases from 15,000-20,000 at the DEJ to 45,000-60,000 at the pulp (77). The diameters of the tubules also show a difference. In the coronal dentin the average tubule diameter at DEJ is 0.5 to 0.9 μm but it increases to 2 to 3 μm towards the pulp. In the root dentin, the deep dentin tubules are more numerous and larger compared to superficial layers (74) (Figure 2.2).

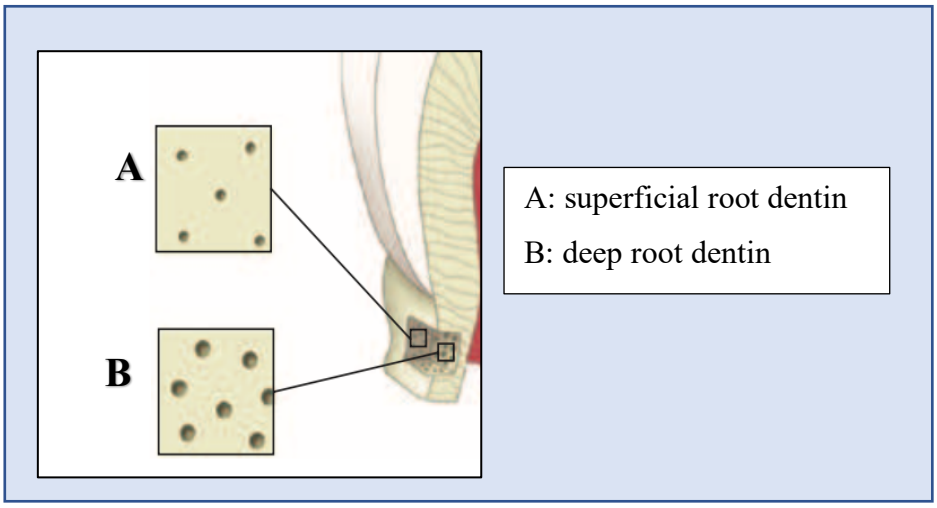


Figure 2.2. Superficial and deep root dentin (74)

Dentin's hardness is about one fifth of the hardness of enamel. Also, dentin found near the pulp is three times softer compared to dentin situated near the DEJ. It should be noted that even though it is a hard-mineralized tissue, dentin it is still flexible. The approximate modulus of elasticity of dentin is 18 gigapascal (GPa) (78). Due to its flexibility dentin supports the more brittle and less resilient enamel. The approximate tensile strength of dentin is 98 megapascals (MPa) which is very high compared to the approximate tensile strength of enamel which is 10 MPa. The approximate compressive strength of dentin is 297 MPa which is less than the approximate compressive strength of dentin which is 384 MPa) (78).

For *in-vitro* and *in situ* dental experiments, teeth obtained from animals have been used as substitutes to human teeth. The most commonly used human teeth substitute over the last 30 years has been bovine teeth compared to other substitutes such as primate teeth (79), swine teeth (80), equine teeth (81) and shark teeth (82). The use of bovine teeth has also increased significantly over this period of time (83). Most of the in vitro studies are performed on root dentin tissue (84-88) due to thin structure of the cementum, different forms of cementoenamel junction overlapping, edge to edge or with a gap (89). The aforementioned factors jeopardize the standardization of the study samples.

2.1.2. Cementum

Cementum is the hard, avascular dental tissue which covers the anatomic roots of teeth. It is structured as a thin layer and has a yellow color slightly lighter than dentin. The cells forming the cementum are called cementoblasts. They originate from undifferentiated mesenchymal cells in the connective tissue of the dental follicle. By weight, cementum is %45-50 inorganic material (hydroxyapatite) and %50-55 organic material (collagen and protein polysaccharides) and water (74).

In the cementum, principal fibers of the periodontal ligament are present. The portions of fibers embedded inside the cementum and the alveolus bone in order to attach the tooth to the alveolus are called *Sharpey fibers* (74).

The formation of cementum continues through life. In order to keep the attachment intact, as the superficial layer ages a new layer of cementum is deposited (74). Between the ages of 11 and 70 the thickness of cementum triples and the most increase is seen in the apical region (90). In fact its thickness in a healthy individual is approximately 20-50 microns near the cervical region of the tooth and 150-200 microns at the root apex (91).

In the coronal half of the root, *acellular cementum* is present and there aren't any cementoblasts present. The apical part of the root is associated with the *cellular cementum.* The apical foramen of the root is surrounded with cementum and cementum also may extend to the inner wall of the pulp canal slightly. If the occlusal or incisal surfaces are worn, cementum thickness on the root apex end may increase to compensate (74).

To a certain limit, cementum can repair itself. Even though it is not resorbed under normal conditions; resorption may take place due to reasons such as excessive orthodontic pressures or very fast orthodontic movements (74).

2.2.Dental Plaque Biofilm

In order to understand periodontal pathogenesis and the rationale behind periodontal treatment, it should be noted that the number of host mammalian cells constituting the human is approximately 10^{11} and there are 100-1000 times more microbiome cells present. (92). It is astonishing to think that the majority of the cells constituting the human body belongs to microorganisms. The "human microbiome" is a highly complex organization. One of the main focuses of the field of periodontology is the microorganisms which are residents of the oral cavity and that determine the periodontal health state of an individual.

The oral cavity is a warm and moist "open ecosystem" populated by many microorganisms (93). The majority of oral microorganisms are bacteria (94). Bacteria which float independently in the saliva are called planktonic bacteria. Once bacteria colonize in biofilm structures adhering to hard surfaces in the oral cavity such as teeth, restorations, dental implants and prosthetic restorations, this bacterial community is called DPB. DPB is the primary etiological factor of periodontal diseases (3,4,94). In order to persist in the oral cavity, bacteria have to exist in a biofilm (93,94) rather than in the planktonic form.

In a biofilm, bacteria exist as members of a polymicrobial community of species that interact (94). In the oral cavity, existing in a DPB has many advantages for microorganisms. It provides physical protection from phagocytosis, resistance against host defenses, enhanced tolerance to antimicrobials, ß-lactamase production by neighboring cells protecting sensitive organisms, synthesis of novel proteins to bind-attach to host molecules, coordinated gene responses by producing cell to cell signaling molecules, having a more efficient metabolism and enhanced virulence (95).

DPB can be defined as "a microbial community that develops on the tooth surface embedded in a matrix of polymers of bacteria and salivary origin" (95). For a biofilm to be established, an ordered series of events take place in the following sequence (5):

First the supragingival DPB is established through the formation of the pellicle which is also called a conditioning film (a layer of acellular glycoprotein material covering all the surfaces in the oral cavity) followed by pioneering micro-organisms settling in the pellicle and multiplying to form colonies. The first colonizers are Gram-positive aerobic bacteria. Two to four days later Gram-positive rods and filaments, Gram-negative anaerobic organisms (*cocci* – filaments) and fusiform bacteria start to colonize. As the DPB matures, both *spirochetes* and spiral forms appear. At this stage the majority of the organism are filamentous. As the anaerobic content of the microbial dental biofilm increases, the pathogenicity also increases (3,4,93,94).

The steps of microbial dental biofilm formation can be summarized as follows (5,93,94):

i) association (*also known as reversible adhesion*): loose association of bacteria to the pellicle through physical forces
ii) adhesion (*also known as irreversible adhesion*): binding of the primary colonizers (streptococci and actinomyces) to pellicle receptors through adhesins followed by other bacteria binding to the primary colonizers (*also known as co-adhesion*)
iii) bacterial proliferation
iv) formation of microcolonies: secretion of protective extracellular polysaccharides such as dextrans and levans
v) formation of an attached plaque *(biofilm formation)*: complex groups are formed by microcolonies and have metabolic relationships
vi) plaque growth and maturation: at this stage the metabolic products such as lipopolysaccharides (LPS) cause host immune system activation

Frist the supragingival DPB is formed followed by the subgingival DPB. The subgingival DPB is established inside the gingival sulcus or periodontal pocket. As the depth of the pocket increases, the number of pathogenic Gram-negative anaerobic bacteria also increase (3,93). Further dysbiosis enables a more periodontally pathogenic flora to be established. Overall in the DPB, there are more than 700 species of

bacteria (94). According to the expanded Human Oral Microbiome Database currently there are a total of 775 microbial species (96) present in the oral cavity.

As the biofilm accumulates, host-immune response is triggered. The flow of gingival crevicular fluid increases to provide host defenses against microorganisms and their byproducts (94). A local rise in temperature may also be measured (94). The increase in gingival crevicular fluid flow may conversely also benefit the obligate anaerobic and proteolytic species of bacteria which are present in the subgingival DPB by providing them with host proteins and glycoproteins which can be used as bacterial cofactors for growth (97,98)**.** This proteolytic process may affect the pH, making the environment suitable for bacteria associated with periodontal diseases at high counts such as: *Porphyromonas gingivalis, Provotella intermedia, Treponema denticola, Tannerella forsythia, Fusobacterium nucleatum (5,6).* Not only the mere presence of these microorganisms but the total counts matter for the dysbiosis (99).

Pie chart areas were adjusted to represent the total counts (7) (Figure 2.3). The gray category represents species which did not fall into a complex of Socransky and may be new species.

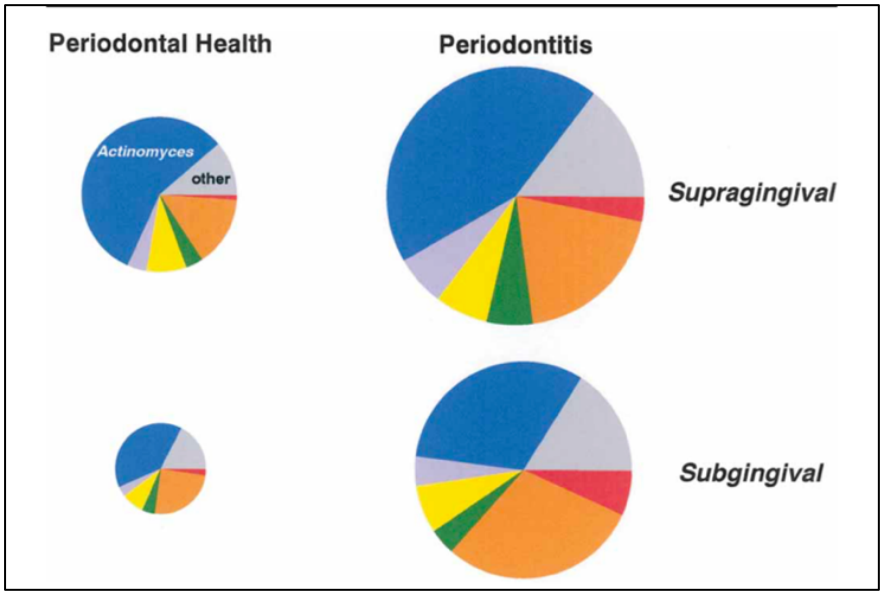


Figure 2.3. Comparison of the microbiota of supra- and subgingival DPB of subjects in health and periodontitis (7)

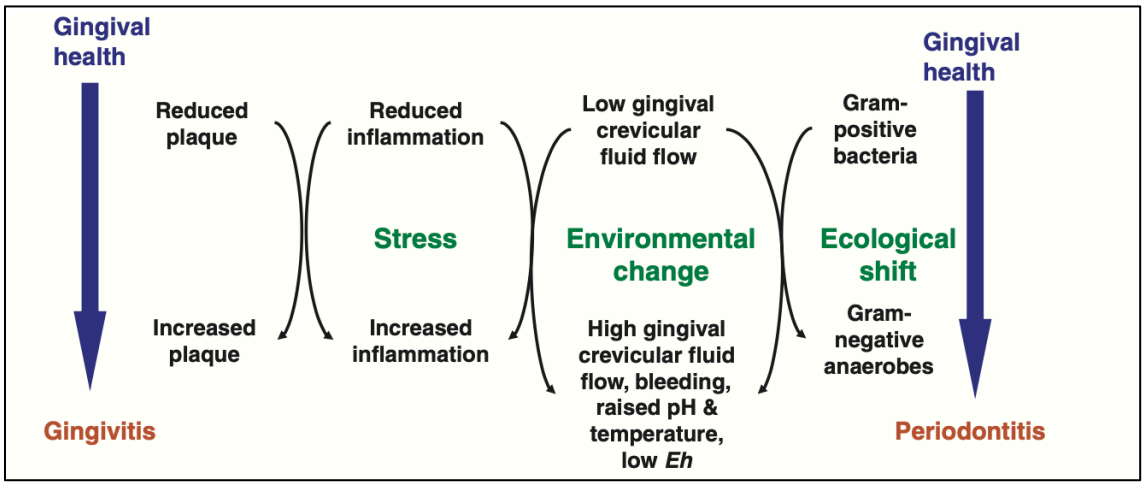


Figure 2.4. Ecological plaque hypothesis (94)

When the ecological plaque hypothesis (Figure 2.4) in relation to periodontal disease is studied (3,94,95,100), it is seen that an accumulation of DPB increases inflammation. As a result of the inflammatory response, local environmental conditions change. Host proteins and glycoproteins are introduced to the environment; high gingival crevicular fluid flow, bleeding, a raise in pH and temperature and a lowered activity of electrons (Eh) is observed. These changes cause an ecological shift from gram-positive bacteria which are associated to gingival health to gram-negative anaerobic bacteria which are associated to disease.

Disease prevention can be achieved by preventing DPB accumulation triggering the inflammatory response and also by interfering with other factors causing the shift (100). Promoting the presence of an oral microbiota at a state of homeostasis (94) is one of the most important responsibilities of all oral health care providers.

2.3.Pathogenesis of Periodontal Diseases

Majority of periodontal diseases are multifactorial, biofilm associated, chronic, inflammatory diseases associated to dysbiotic DPB (1). For a periodontal disease to occur, the symbiotic interplay between the host factors and the microbial biofilm has to turn into a pathogenic form.

Bacterial species present in the oral cavity continuously interact with the host immune system. Once dysbiosis takes place and periodontally pathogenic bacteria multiply in number, cascades leading to disease may be triggered. Through the release of a wide range of inflammatory cells including cytokines, chemokines and pro-inflammatory mediators; periodontal structures including the attachment and alveolar bone may be destructed (3,4).

On a biological level, a neutrophilic infiltrate is present even with gingival health since bacteria are always present in the oral cavity (101). However, from a clinical standpoint a patient is considered to be gingivally healthy when the inflammatory infiltrate and the host response are consistent and at a state of homeostasis (102). The nature of the relationship between the host and microorganisms is symbiotic in nature at this stage. The host response is both proportionate and resolving (103).

When dysbiosis occurs and the homeostatic balance is lost; first gingivitis develops. Gingivitis is a non-specific inflammatory condition resulting from the sustained DPB (104). Gingivitis induced by the DPB at the site level is defined as: “an inflammatory lesion resulting from interactions between the DPB and the host's immune-inflammatory response, which remains contained within the gingiva and does not extend to the periodontal attachment system (cementum, periodontal ligament and alveolar bone). Such inflammation remains confined to the gingiva and does not extend beyond the mucogingival junction and is reversible by reducing levels of DPB at and apical to the gingival margin” (2).

In gingivitis, a threshold of DBP accumulation is required to induce gingival inflammation. This threshold level and how it affects the rate of disease progression is inconsistent for different individuals. The variance between individuals is due to local risk factors (predisposing factors) and systemic risk factors (modifying factors) (2).

Local risk factors encourage plaque accumulation (102) at specific sites either through inhibition of plaque removal and/or by creating a plaque accumulation encouraging niche (104). DPB retention factors include tooth anatomical factors and

subgingival restorations (67,70) which enable both adherence and maturation of the plaque. In these areas mechanical plaque removal is hard to accomplish.

Systemic risk factors influence the immune-inflammatory response to DPB by causing an exaggerated inflammatory reaction (2). These factors include smoking, metabolic factors, nutritional factors, pharmacological agents, elevations in sex steroid hormones and hematological conditions (2).

It has been shown that the sites which progress to attachment loss persistently show higher levels of gingival inflammation (105)**.** Gingival inflammation is a major risk factor and a pre-requisite for periodontitis and progressive attachment loss (106).

In patients who are not susceptible, disease progression further than gingivitis does not take place, however, in susceptible patients an inappropriate and excessive host response may be triggered leading to periodontitis (3).

Excess cytokines, reactive oxygen species, and matrixmetalloproteinases are produced. Due to their interaction with their antagonists, collateral tissue damage takes place. Tissue damage causes a release of damage-associated molecular peptides which further propagates the inflammatory response. Innate mechanisms which are responsible for resolving the inflammation fail and as a result inflammatory lesions become chronic (3).

Periodontitis is the 6th most prevalent chronic inflammatory disease of mankind that causes the tooth-supporting apparatus to be destructed progressively (107). Loss of periodontal tissue support (expressed as clinical attachment loss, radiographically assessed alveolar bone lose, presence of periodontal pockets, gingival bleeding) has a high prevalence, and it may lead to the disturbance of function and disability through the loss of teeth (108).

There are complex, interdependent relationships both between the DPB-host response and also within the DPB as explained above. The classical periodontal disease pathogenesis model explaining this interplay was developed by Page & Kornman (4) in 1997. The most recent scientific findings have caused the classical model of

periodontitis to change. The contemporary model of host-microbe interactions covers host-microbe interactions in the pathogenesis of periodontitis (3) (Figure 2.5).

The rationale of non-surgical periodontal therapy and supportive periodontal care (SPC) is to mechanically remove the DPB and root surface contributing factors which are the primarily considered as important etiological factors of periodontal diseases.

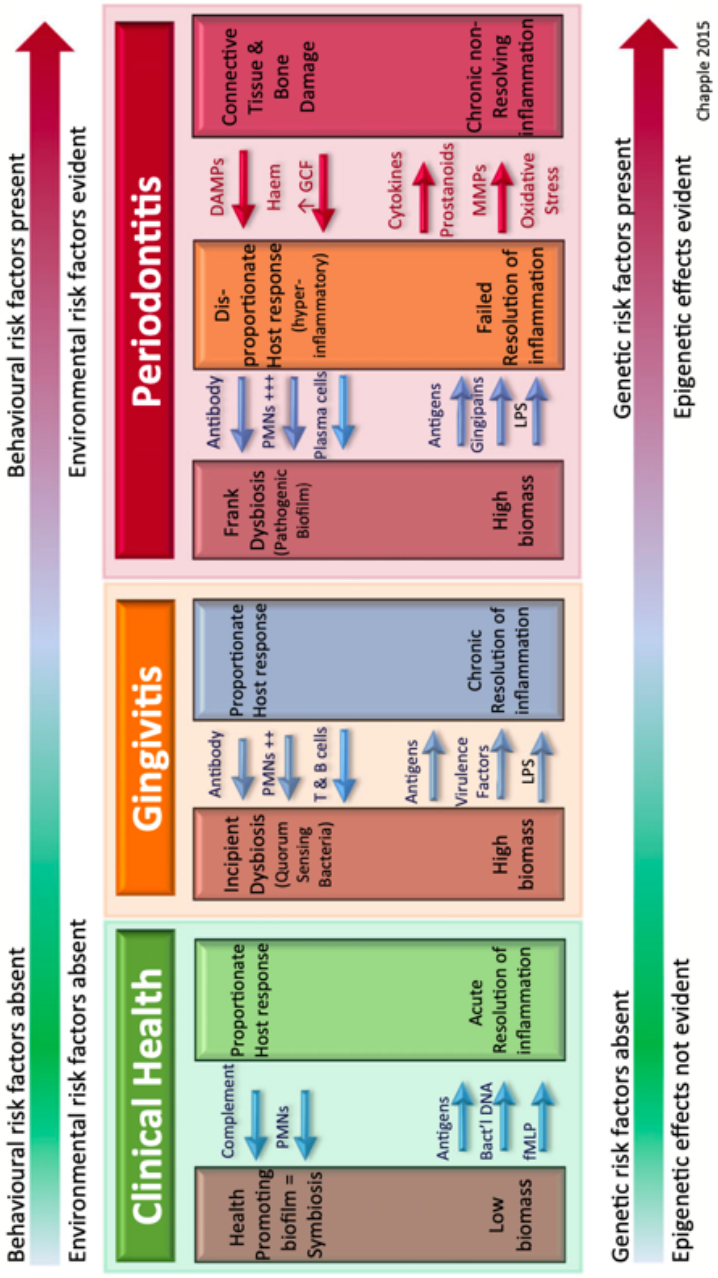


Figure 2.5. Contemporary model of the pathogenesis of periodontitis (3)

2.4. Mechanical Periodontal Therapy

2.4.1. Non-Surgical Periodontal Treatment

Non-surgical periodontal therapy aims to remove the DPB, calcified accretions and other etiological factors related to periodontal diseases from root surfaces and the subgingival sites in order to provide and preserve periodontal health (9,10).

"Professional mechanical plaque removal" is the term used to describe the professional interventions required to remove supragingival plaque, calculus, and other plaque retentive factors. (1). In addition to professional mechanical plaque removal, interventions aimed at removing/eliminating the subgingival DPB and calculus may be needed during non-surgical therapy.

Even though it was previously believed that the contaminated root cementum had to be scaled and planed for the elimination of bacterial products such as endotoxins and LPSs (109), current literature has proven that the week adherence of bacteria and its products to the root surface causes such an intervention to be considered as an unnecessary overtreatment (110-114). This approach has also caused the term "root planing" to change into "subgingival debridement" which has been further changed into "subgingival instrumentation" in the latest clinical guideline (1).

Reducing the number of gram-negative anaerobic organisms is the essential step of non-surgical periodontal therapy. Even though many patients apply to clinics raising their concerns about the presence of self-detectable calculus; studies have shown that even in the presence of calculus, healing will still take place if subgingival DPB is eliminated (115). After therapy, patients should be educated about the importance of self-performed oral hygiene practices, the importance of DPB removal to reduce total bacterial load and the fact that interventions may be necessary even in the absence of calculus. The prevalent patient perception defining non-surgical therapy as a "simple removal of calculus" should be changed. Raising awareness on periodontal health may increase self-

diagnosis accuracy of early inflammatory signs such as gingival bleeding and adherence to periodontal maintenance (116).

It should be noted that a total removal of biofilm from the oral cavity is impossible (117-120). Still subgingival instrumentation is the method of choice and it is accepted as the gold standard (121) for the treatment of many periodontally compromised patients. In fact, a disruption in the ecology of the biofilm is achieved by both supra and subgingival instrumentations since they are interrelated regarding the microbial contents of the DPB. Following this disruption, a shift towards a more host-compatible microflora takes place enabling the host immune system to become capable of coping with the remaining bacteria. The result is either a closure of the subgingival pocket and/or improved clinical parameters with the elimination of inflammation (gain in clinical attachment level (CAL), probing pocket depth (PPD) reduction, reduction in bleeding on probing (BoP)) (9,13,121).

Many different instruments can be used during non-surgical periodontal therapy. Manual debridement can be achieved by using hand instruments. These tools include curettes, scalers and hoes (9). Debridement can also be done by adopting a machine-driven approach. Power-driven instruments including sonic scalers, ultrasonic scalers (USs) and the LOD can be used. In addition, APs are also currently available on the market as alternative tools for removing DPB from teeth as well as extrinsic stains.

2.4.2. Supportive Periodontal Care

When treating patients with periodontal diseases, the statement used by the community of periodontists should always be remembered and explained to the patient: "*Once a perio patient, always a perio patient*.". Since periodontal diseases are chronic diseases, even though they may be treated with non-surgical periodontal therapy and surgical interventions when needed; a SPC regimen based on mechanical approaches is always required to keep the disease at its passive state, sustain the successful long-term results from the treatment, prevent tooth loss and "assist the patient in maintaining oral health" (115).

During the SPC appointment the medical and oral histories of the patient are taken, both intraoral and extraoral examinations are conducted. The oral cavity is examined and evaluated as a whole in order to locate and treat conditions and diseases found in the oral cavity. Restorative and prosthetic factors are evaluated during the examination as well. In order to prevent recurrence/progression of periodontal disease, prevent tooth/implant loss; DPB and contributing factors are removed, patients' oral hygiene practices are evaluated, and oral hygiene instructions are provided and repeated (1,122,123).

During SPC, DPB control is essential. The latest guideline suggests routine professional mechanical plaque removal to be performed during SPC to limit the rate of tooth loss and provide periodontal stability and improvement (1). Studies show that compliance to SPC increases tooth survival in patients (124,125)

Selection of the appropriate SPC interval is especially important based on the oral hygiene levels and the efficacy of previous mechanical instrumentation. Risk factors of each individual patient are used for the determination of the interval. Both systemic factors (diabetes, smoking etc.) and local factors (plaque retentive areas, faulty dentistry etc.) should be assessed (126). In one of the most common models: periodontal risk assessment (126) patients are grouped according to their risk profiles. Low-risk patients have a 12-month SPC interval, moderate-risk patients have a 6-month SPC interval and high-risk patients have a 3-month SPC interval.

In conclusion, it should be remembered that patients should always be kept under surveillance lifelong after the completion of their active periodontal treatment. The risk factors for periodontal disease should also be controlled to prevent the recurrence/progression of periodontal diseases. With appropriate SPC through mechanical approaches, the periodontal health status achieved can be maintained for up to 20 years in some patients (115). During SPC, patient dental history should be questioned carefully since local risk profiles are dynamic and can change, also changing the required SPC intervals.

2.4.3. Instruments Used in Professional Mechanical Periodontal Therapy

Hand instruments and power-driven instruments can be used during mechanical periodontal therapy. Hand instruments have sharp working tips which break the bond between the tooth surface and the deposit. Hand instruments are preferred by many clinicians since they are believed to offer the clinician direct tactile control during instrumentation (127). Power-driven instruments on the other hand are argued to be easier to use and time saving compared to hand instruments (20). Even though the choice of instrument is a personal decision, there are studies that show that more clinicians prefer power-driven instruments over hand instruments (20).

The latest clinical guideline (1) recommends the use of hand instruments or power-driven instruments either alone or in combination during subgingival periodontal instrumentation according to the individual needs of the patient. It has also been stated that the patient autonomy should always be respected by the clinician while instrument choice is being made.

2.4.3.1. Hand Instruments

Hand instruments provide tactile sensitivity, better visibility, safe bacterial elimination in comparison to aerosol and heat generating devices. For hand instrumentation to be effective; adequate access, good adaptation, effective angulation and complete root coverage with strokes should be achieved (128).

Curettes, sickles, hoes, files and chisels have been used for scaling and root debridement. Throughout the historical evolution the commonly used material in hand instruments is stainless steel. High-carbon steel instruments are also available and found to be superior by some clinicians (123). Sickle scalers and curettes are the most commonly used hand instruments. Sickle scaler is used for supragingival calculus removal and curettes are mostly used subgingivally (129). Amongst these instruments, curettes are accepted as the most versatile and effective instruments (118,130,131).

There are two basic types of curettes which are the Universal Curettes and Gracey Curettes. The differences between these curettes are (22):

-Area-specificity
-Number of cutting edges
-Curve of the cutting edge
-Angle of the face to the terminal shank

Universal Curettes are not area specific. The double ended instrument is designed to be able to adapt to all tooth surfaces. Since the face of the blade is honed at a 90°angle to the lower shank, both cutting edges on each blade can be used (128). Examples to Universal Curettes are Columbia curettes and Gothenburg curettes (129).

Gracey Curettes are area specific. Different curette designs are available to be used in specific anatomic areas. Gracey Curettes may be considered the best instruments for subgingival scaling and root planning since they provide the best adaptation to the complex root anatomy (123)**.** The offset blade angulation of these curettes is 70-80°. This angulation enables the proper angulation of the blade to be achieved when the lower shank is positioned parallel to the tooth during instrumentation (128).

In addition to these two basic types of curettes there are also extended shank instruments (ex. After Five Curettes – Hu-Friendy Co.) and mini bladed instruments (such as Mini Five Curettes - Hu-Friendy Co.).

After Five Curettes have a terminal shank which is 3mms longer to allow an accessibility into deeper pockets which are 5mm and more. These curettes have a thinned blade to enable to curette to be inserted smoothly into the subgingival area and a large-diameter tapered shank. They may be used for the deep pockets of maxillary and mandibular posterior teeth (128).

Mini Five Curettes' blades are thinner and half the length of standard Gracey Curettes. Their terminal shanks are also 3mms longer than standard Graceys (128). Their

design allows access to deep narrow pockets, furcations, developmental depressions, line angles; deep-tight pockets on facial, lingual and palatal surfaces. They can also be used in areas where standard Gracey Curettes and After Fives cannot be inserted fully due to root morphology or the presence of a tight tissue with minimal tissue distention. In such areas Mini Five Curettes can be used without causing tissue trauma (128).

Even though aforementioned curettes are the most commonly used ones, new curettes with distinct features and novel designs are being manufactured and placed on the market.

For the efficient use of hand instruments, they require re-sharpening frequently (21,24). After being used for several strokes, a decrease in instrument sharpness and effectiveness has been shown in many studies (21,24,132,133). However, only a very small minority of clinicians sharpen their instruments every 5 to 20 strokes (133). Even though it is fundamental to work with a sharpened instrument to achieve a clean and smooth surface, sharpening procedure is very unpractical and it damages to the instrument by changing the original contour, weakening it, causing breakage during function and creating harmful metal tags (22).

It was also shown that as the cutting edge of the instrument gets duller, application of more force is going to become inevitable (26). The requirement to make additional movements and apply more force will not only be time consuming for the dentist but it will also cause discomfort to the patient and make the clinician seem “heavy handed” (134). The clinical outcomes of sharp and dull instruments are summarized on Table 2.1.

Table 2.1. Clinical outcomes using sharp versus dull instruments (135)

Clinical Outcomes Using Sharp Versus Dull Instruments		
Outcome	Sharp Instrument	Dull Instrument
Tactile sensitivity	Increases	Decreases
Client safety and comfort	Increases	Decreases
Working efficiency	Increases	Decreases
Control	Increases	Decreases
Lateral pressure	Decreases	Increases
Probability of burnished calculus	Decreases	Increases

To overcome this problem instruments with "edge retention" properties were recently introduced to the market. Manufacturers claim that these instruments need either little or no sharpening yet still allow unproblematic maintenance and a long-term display of effectiveness (22). Different metal alloys and different coatings are made out of different materials such as specially filtered titanium nitride/stainless steel (XP Technology American Eagle) or *Diamond Like Carbon* and metal nitrite coatings (136) (LM Sharp Diamond).

2.4.3.2. Power-Driven Instruments

Power-driven instruments are relatively blunt compared to hand instruments and the acceleration of the vibrating tip is relied upon for the disruption of DPB and calculus (11,123). The physical factors that play a role in the action mechanism of power instruments are frequency, stroke and water (liquid) flow (123).

During instrumentation, water is used as a coolant to reduce heating caused by friction (129). Currently there are new power-driven instruments on the market with liquid reservoirs which can be filled with the lavage solution of the clinicians' choice. The physiological effects of water may contribute to the efficacy of the instrument which are: acoustic streaming (unidirectional fluid flow caused by ultrasonic waves) (137), acoustic turbulence (movement of the tip accelerating the coolant and producing an intensified swirling effect) (123) and cavitation (bubble formations in water caused by high turbulence which implode and produces shock waves in the liquid that creates further

shockwaves throughout water) (138). The combination of these factors was shown to disrupt the DPB *in vitro* (137-141).

Power-driven instruments are classified into sonics and USs. USs may either be magnetostrictive or piezoelectric (123,129). Oscillation generator generates high vibrational energy which is conducted to the scaler tips in all these instruments.

Magnetostrictive scalers contain either a stack of flat metal strips or a rod of ferromagnetic materials to act as the transducer (129). When electrical energy is applied, these stacks or metal strips change dimension. Vibrations travel from the stacks/metal strips to the working tip (123). The movement of the tip is elliptical or circular (129). This movement provides 4 active working surfaces for the tip. The frequency range is between 18,000 to 50,000 Hz (123).

Piezoelectric scalers contain ceramic discs in the handpiece and these discs change dimension as electrical energy is applied (123). The movement of the piezoelectric scaler is primarily linear in direction (129). This movement provides 2 active working surfaces for the tip. The frequency range is between 18,000 to 50,000 Hz (123).

Sonic instruments on the other hand utilize mechanical vibrations of the working tip. Compressed air from the dental unit makes the tip vibrate with frequencies ranging between 2,000 to 6,000 Hz (101). Tips of sonic instruments have a mainly elliptical working motion (102).

A comparison of the advantages and disadvantages of power-driven instruments as compared with hand instruments are summarized on Table 2.2.

Table 2.2. A comparison of the advantages and disadvantages of hand instruments and power-driven instruments (135)

Advantages and Disadvantages of Power Instruments as Compared with Hand Instruments	
Advantages	Disadvantages
-Increased efficiency -Multiple surfaces of the tip capable of removing deposits -No need to sharpen -Less chance for repetitive stress injuries -Large handpiece size -Reduced lateral pressure -Less tissue distention -Lavage and irrigation with antimicrobial agents and cavitation -Acoustic microstreaming	- More precautions and limitations -Client comfort (vibration and noise) - Aerosol production -Temporary hearing shifts -Less tactile sensation -Reduced visibility

There are many different tip designs available (Figure 2.6) besides the new ones being designed, manufactured and marketed. In addition to standard tip designs which are considered to have a universal design with large diameters; modified power-driven scaler tips are available. There are also tips available for the removal of heavy supragingival calculus (123). Some design and surface modifications are made to provide site specificity; use in subgingival areas, furcation areas and around implants. Tips with right and left contra angulation are used to gain better access and adaptation to root surfaces (123).

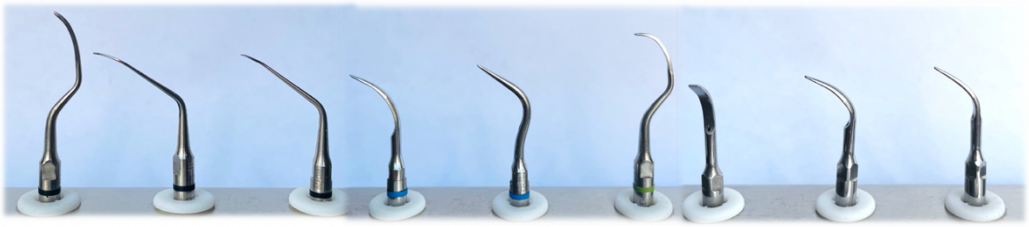


Figure 2.6. Various tip designs for ultrasonic instrumentation

- **The Linear Oscillating Device**

A more recent power instrument on the market is the LOD. Horizontal vibrations of the device are converted into vertical vibrations by a resonating ring. As a result, the working tip moves parallel to the tooth surface. The ultrasonic oscillations are at a frequency of 25 kHz. A suspension of hydroxyapatite particles and water also play a role in the transmission of energy to the root surface (142). There are different tip designs available to be used during periodontal treatment.

Even though it was cited "as a significant progress in power-driven instruments" the vibration mode of this system is similar to the vibration mode of USs (143). The oscillation frequency is also similar to ultrasonic frequencies. The tip is set at right angles to the longitudinal oscillation of the handpiece. The tips of USs are similar in terms of these motions as the tip of these scalers curves towards a right angle to the main driver (143). The clinical and microbiological outcomes of the LOD was found comparable to conventional hand and power-driven instrumentation, yet it was found less efficient for removing large calculus (27).

Braun et al. (28) reported that the use of LOD causes a reduction in the pain experienced by the patient during treatment. Power settings of the LOD and US have not been specified in the study. Twenty patients were included. Three teeth with comparable periodontal parameters were treated with 3 different methods in each patient: instrumentation with hand instruments, a conventional US and LOD. A manometer was used during instrumentation and patients were told to set the pressure in proportion to their perceived intensity of pain. Manometer was videotaped and the videotape was evaluated in intervals of 1 second. Also, after the treatment the Visual Analog Scale (VAS) was used to measure the amount of pain experienced by the patient. It was concluded that the LOD caused less discomfort and pain and it was stated that this may increase compliance to non-surgical periodontal therapy and SPC.

An in vitro study of Kishida, Sato & Ito (144), compared the LOD with the conventional piezoelectric US and Gracey curettes. This study concluded that when both

power instruments were set at a medium power setting, profilometric analysis of the roots revealed smoother surfaces being produced by the LOD, however they have also concluded that the LOD was the slower in calculus removal compared to piezoelectric US.

Kocher et al. (145) found that there was no difference between the discomfort levels of patients when LOD was compared with a conventional US used at a low power setting. Power setting of the LOD was not specified. In this study 38 maintenance patients with 3-month SPC intervals were included in the study. Patients were required to have at least 2 teeth with a PPD greater than 4mm and at least 15 teeth in total. Patients were assigned to be treated with either the LOD or a conventional US. BoP, PPD or CALs were improved in both groups at the 6^{th} month. No difference was found between the 2 treatment modalities. The pain intensity perception of the patients also did not differ.

Hoffman et al. (29) compared the discomfort levels caused by LOD with a conventional piezoelectric US. It was concluded that during SPC, LOD provided less discomfort, however inconsistent power settings of the instruments (conventional US: maximum power / LOD: %70) should also be taken into consideration when the results are reviewed.

Braun et al. (32) evaluated the efficiency of subgingival calculus removal with the LOD (with the metal probe and curette tips) versus conventional US and Gracey curettes in an *in vitro* study. Two different fluids were available (polishing fluid and abrasive fluid) to be used with the LOD when the study was conducted. These two fluids were compared as well. Sixty extracted human teeth with calculus on the root surface were placed in an artificial periodontal pocket model. Root surface photographs were taken at intervals of 10 seconds and the removal of calculus was assessed with a surface analysis software. Hand instrument was found to be the most efficient instrument, LOD with the metal probe tip was less effective compared to curette tip regardless of the type of fluid used. When the metal probe tip was used with the abrasive fluid, less calculus was removed compared to the curette tip used with the polishing fluid. LOD was found to be

equally efficient compared to the conventional US, when the curette tip and the abrasive fluid were used together.

Braun et al. (33) in another study, compared root substance removal of the LOD with the conventional US and hand instruments. Instrumentation with the LOD was conducted in 2 groups. The first group used the polishing fluid with the metal curette tip and the second group used the abrasive fluid instead of polishing one. Root surfaces were treated for 12 minutes in an artificial periodontal pocket and at intervals of 2 minutes and removal of hard tissues was assessed with a three-dimensional scanning device. The root material removal with the conventional US and the LOD together with the abrasive fluid were found to be more compared to other 2 groups. Root surface removal with hand instrumentation and by using the LOD with the polishing fluid was found to be similar compared to one another. Authors concluded that hand instrumentation or the LOD with the polishing fluid may be used for root surface instrumentation to avoid extensive removal of root substance.

There are other studies available comparing the LOD with conventional methods in terms of surface characteristics, inflammation, hypersensitivity, microbial load etc. (30,31,34,146). It should be noted that the power settings chosen for both the LOD and the conventional USs are not standardized between the studies.

The LOD can currently be used with an aqueous solution containing hydroxyapatite[2] as only solution available on the market. During instrumentation, this polishing fluid is diluted with water to the ideal working concentration. The manufacturer claims that the use of this solution enables a minimally invasive treatment of periodontitis and the use of this solution plays a role in the gentle but effective removal of the DPB, supra and subgingival calculus, bacteria and endotoxins (147).

Another study by Braun et al. (148) evaluated the effect of the aforementioned polishing fluid in terms of morphological alterations on the root surfaces. Thirty freshly extracted human teeth were used in this study. Root surfaces were planed, and citric

[2] **Vector™Fluid Polish,** Dürr Dental, Bietigheim-Bissingen, Germany

acid was used to remove the smear layer. For the group treated with the polishing fluid it was claimed that the dentinal tubules were covered by a granular layer suggesting an anti-hypersensitivity effect. To the best of our knowledge, currently there aren't any other studies conducted exclusively on the polishing fluid focusing on its effect in removing DPB and altering SR in the literature.

2.4.3.3. Air Polishing

The objective of air polishing (also known as injection abrasive water jets) is removing the DPB in a highly efficient and convenient way (31). They are also beneficial in removing extrinsic stains (149). The first AP used for stain and DPB removal was introduced in the late 1970s. APs can be used as alternatives to curettes, sonic and USs (46) in some cases, since they allow the removal of DPB (150).

The AP system is composed of a light handpiece that generates a slurry of pressurized air, APP and water (149). The powder of choice is placed inside a chamber. The chamber is stirred up by pressurized air. A flow of air and water is simultaneously transported to the tip of the nozzle (150). The interaction of solid particles with the treated surface is the basic event in the abrasive process (151). The efficacy of APs is influenced by hydropneumatic parameters, abrasive media related parameters and user-dependent parameters (152,153). The hydropneumatic parameters are the amount of water and air pressure. Both an increase in the water settings and pressure were shown to increase the efficacy of the instrument (154,155). The abrasive media related parameters are the mass of the emitted powder, grain size, shape, geometrical form and hardness. A greater particle mass, size, hardness and angularity increase the abrasiveness of the slurry (151,152,156-158). User-dependent parameters are the stand-off distance between surface and nozzle, angulation of central beam, instrumentation time and the type of the treated surface. It has been shown that the depth of defects that arise due to instrumentation will decrease if the working distance between the nozzle and tooth surface increases (155). Even though the angulation between the nozzle and the surface plays an important role in the transfer of energy between the particles and the surface (157), the short working distances in the dental setting may be reducing or masking the effect (150). The angulation of AP relative to the treated surface is still an

ongoing debate since there are studies available where no change has been shown between the surface removal when the angulation of the nozzle was 45° or 90° to the treated surface (155). The instrumentation time has a strong and significant impact on the efficacy of the device (38,155,158).

In addition to these factors, it is important to know that the design of the device also plays a particularly important role in influencing the abrasive properties. It has been shown that even small differences in nozzle (dimension changes, diameter of openings, tube length, tube curvature) change the efficacy of the instrument (152,158).

The use of APs previously, was limited to supragingival application and mostly for stain removal (135). Introduction of a new subgingival nozzle (35) and of new less abrasive APPs with a enabled a high efficiency in periodontal treatment (45,46).

- **Air Polishing Powders**

APPs are essential for air polishing. Petersilka et al. (44) have shown in an *in vitro* study that using an AP device without an APP and only with water does not remove DPB. However, it should be known that the choice of powder (grain size – crystal shape) is very important since it affects both DPB removal and surface defect formation after application (44).

Currently there are many different APPs available on the market. In addition to conventional $NaHCO_3$ powders (150); aluminum hydroxide, calcium carbonate, calcium sodium phosphosilicate, glycine, erythritol and trehalose APPs can be used. Among these APPs, $NaHCO_3$ is the most well-known large particle APP, proven to damage the tooth surfaces (38,159) and restorative materials (160,161). To compensate for the disadvantages, gentler and finer APPs are introduced such as glycine (36,37,45,46,150), erythritol (39,42,43,162-164) and trehalose (40,41,47,48,165-167).

Trehalose is a disaccharide occurring in nature and it is known as the “cell protector” of many animal and plant species. Many organisms have pathways to produce trehalose

before starting life cycles where they will be exposed to heat, dehydration and freezing (Birck 1963, Clegg 1965, Elbein 1974, Womarsley and Smith 1981, Newman 1993, Paiva and Panek 1996 as cited in 167). There are only a limited number of studies on intolerance to trehalose and currently it is known to be less common compared to lactose intolerance (Bergoz 1971, Dahlqvist 1974, Bergoz et al. 1982 as cited in 167). It has been shown that in addition to being lowly-cariogenic, trehalose may also be anti-cariogenic (166). On market there are two different sizes available for trehalose to be used as APPs.

Animal studies have shown that trehalose inhibits LPS induced production of pro-inflammatory cytokines interleukin-1β and tumor necrosis factor-α in peritoneal macrophages of mice (47) and inactivates the receptor activator of nuclear factor kappa beta (RANKL) inducing pathway in rats and suppresses osteoclast differentiation (48).

Kruse et al. (40) evaluated the application of subgingival trehalose APP during 44 SPC patients with a split-mouth design. Single rooted teeth were chosen for the study. Residual pockets (5mm PPD with BoP or PPD > 5mm with or without BoP) were debrided subgingivally either with trehalose APP or with a sonic device. The primary outcome was the reduction of PPD after 3 and 6 months. The level of discomfort was also measured with the VAS. The reduction in PPD, CALs and BoP was statistically significant for both groups at 6 months. No significant intergroup differences were seen. Compared to sonic scaling, lower incidence of patient discomfort was reported for AP whereas comparable clinical outcomes were reached in both.

In a more recently pilot study by Kruse et al. (41) which was part of the aforementioned study (40), microbiologic analyses were conducted. For this study subgingival DPB samples were taken from 10 participants at the baseline, 3 months and at 6 months. Bacterial counts were determined, isolated bacteria were identified. Bacterial counts were reduced immediately after the treatment and remained low in both groups. AP group showed a higher decrease in the diversity of the detected species compared to the sonic group. Gram-positive anaerobic rod proportion was lower in the AP group (AP %7, sonic instrumentation %25.9). At 3 months the reduction of gram-negative anaerobic rods for the AP group was greater than the sonic group (AP -0.91 Log10 colony forming

unit CFU)/ml, sonic instrumentation -0.23 Log10 CFU/ml p=0.020). Even though a difference in the distribution of aerobes and anaerobes was seen at 6-months, this difference was not statistically significant.

To the best of our knowledge, currently the number of studies available on trehalose APP are very scarce and it is open to investigation since the preliminary findings are encouraging.

2.5. The Relationship Between Surface Characteristics and Periodontal Health

SR is the variation in the height of a surface relative to the reference plane. It may be measured along a single line profile or along a set of surface maps. It is characterized by one of the two statistical height descriptors according to American National Standards Institute and the International Standardization Organization. One of those descriptors is roughness average (Ra) (168). Ra can be defined as "the arithmetic mean of the absolute values of the roughness profile ordinates" (169).

Most of the disease forming organisms present in the oral cavity can only survive if they adhere to non-shedding hard surfaces. Since teeth are the only natural non-shedding surfaces in the human body, they provide a "secure haven for microbial persistence" (8). It has been shown that the SR plays a key role in the bacterial retention processes (51-63). Controlling the SR variable is considered as an important factor in preventing periodontal diseases. It has been shown that the oral bacteria are less likely to colonize on smooth surfaces (170). As an endpoint for periodontal therapy, a smooth-root surface has always been desired by clinicians. Many different approaches were suggested as the last step of root surface instrumentation including the use of various rotary instruments and rubber cups for polishing. A smooth surface was also accepted as a clean surface by clinicians (9).

SR impacts biofilm formation in several ways according to the review of Teughels et al. (171). Bacteria initially adhere to places where they are protected from shear forces and may proceed from reversible to irreversible bonds. As the SR increases, the area

available for bacteria to adhere increases by a factor of 2-3. Since rough areas are not easily cleaned, the DPB in these areas may re-grow by multiplication of the remaining species instead of recolonization (172).

The animal study of Waerhaug (63) conducted on dogs in 1956 questioned whether a rough surface placed below the level of the gingival margin would prevent the healing process and act as a permanent irritant. It was concluded that the presence of bacteria and their toxins caused irritation, rough surfaces facilitated the retention of bacterial plaque and restorations placed below the gingival margin had to be well polished. This study also stated that the enhanced plaque accumulation resulted in the formation of subgingival calculus.

An *in vitro* study by Swartz et al. (61) (1957) compared the accumulation and retention of debris on smooth and rough surfaces in a quantitative manner. Enamel was subjected to an environment where 24 hours old culture was present and incubated for 3 hours. The number of bacteria collected and retained on abraded and polished enamel was evaluated. Results showed a higher bacterial accumulation on the rough surface compared to a smooth one. By brushing the rough surface rigorously to increase the SR and repeating the incubation, it was observed that the rough surface was retaining the bacteria more readily. It was concluded that more bacteria accumulated on a rough surface at a higher rate compared to a smoother one.

Turesky et al. (62) (1961) did an *in vivo* study to compare the early calculus formation in adults by placing smooth and roughened strips in the oral cavity. It was observed that calculus accumulated faster on roughened strips compared to smooth strips. It was also seen that the wiring of a roughened strip to a tooth caused calculus formation.

Rosenberg et al. (60) (1974) studied how the root SR changed after periodontal therapy and the effects of this change on plaque accumulation and gingival inflammation. In this study, even though a statistically significant difference in SR was observed between the groups (curette, US, control); there wasn't a statistical difference in plaque accumulation or inflammatory indices. However, the authors emphasized that the oral

hygiene of the subjects in this study was poor (mean plaque score 2.90) and the ability of smooth roots to enhance plaque control may have been overlooked.

As the number of studies on SR and plaque accumulation increased, research was also conducted on dental materials commonly used in the oral cavity at the time. Budtz-Jörgensen and Kaaber (52) (1986)studied the changes in plaque accumulation and denture stomatitis occurrence when the fitting denture surface was coated with a glaze. In this study, half of the denture was glazed, and the other half was not glazed. 1 week later, more DPB presence was measured on the rough, unglazed surface of the denture concomitant with the high number of CFU.

Quirynen et al. (58) (1990) studied the relative effects of surface free energy and SR on the growth of DPB. 2 strips were placed on the central incisors of 16 volunteers. One strip was fluorethylenepropylene (FEP), the other strip was made out of cellulose acetate (CA) and the third surface was the tooth surface. The surface free energy of FEP has been recorded to be lower than CA (20 versus 58 erg/cm^2). Each strip was divided into 2 parts: a smooth part (Ra ± 0.1µm) and a rough part (Ra ± 2.2µm). The smooth tooth surface on the other hand revealed a surface free energy of 88 erg/cm^2 and a SR of Ra ± 0.14µm. Patients abstained from oral hygiene practices. The DPB formation on the strips were followed for 6 days and evaluation was also done on the 3rd day. At the end of the study period samples were taken from the strip and the tooth surfaces. On day 3, significantly more DPB accumulation was observed on rough surfaces, however surface free energy did not influence the accumulation. On the 6th day, it was observed that the surface free energy also influenced the DPB accumulation, however the influence of SR was found to be more prominent on both accumulation and the composition of the DPB (the percentage of coccoid cells were high on smooth surfaces and low on rough surfaces).

A study regarding the influence of SR in the oral cavity was published by Quirynen et al. (59) in 1993. In this *in vivo* study, 2 abutments of fixed prosthesis supported by endosseous titanium implants were replaced with either by an unused standard abutment or a roughened titanium abutment. DPB samples present on both abutments were evaluated after 3 months. During the experimental period, patients

continued their habitual oral hygiene practices. *P. gingivalis* was only detected on rough surfaces by the DNA probe. When subgingival DPB was scraped from the surface, significantly more (25 times more) DPB was collected from rough abutments compared to the smooth ones. Even though the difference was minimal, lower concentration of coccoid cells were observed on rough abutments. It was concluded that a more complex microbiota with higher percentage of motile organisms and lower percentage of coccoid cells were always harbored by rough abutments. These results were stated to be possibly related to the reduced ability of patients to clean rough surfaces.

An animal study by Leknes et al. (56) published in 1994 evaluated the extent of subgingival colonization of root surfaces instrumented either with a rotating diamond point or by a sharp curette. It was concluded that the smoother curette instrumented surfaces, promoted less subgingival DPB formation compared to diamond treated rougher surfaces. This study demonstrated that the SR caused by subgingival instrumentation had a significant influence on subgingival colonization. Another animal study by Leknes et al. (55) (1996) evaluated the gingival tissue reactions facing root surfaces instrumented either with a rotating diamond point or by a sharp curette. The results have shown that the gingival inflammatory reaction was significantly affected by the modality of the subgingival instrumentation and it was most likely due to a difference in subgingival DPB formation. The authors have concluded that SR caused by periodontal instrumentation affected gingival inflammatory reactions.

A review of the literature (172) conducted on 1995 revealed that rough surfaces promote plaque formation and maturation. Even though surface free energy is also important (it collects more bacteria, makes the plaque bind stronger and selects specific bacteria), SR has been shown to be more important. In order to prevent supragingival DPB formation which will eventually be responsible for periodontitis, surface characteristics should be carefully taken into consideration. The same review evaluated many *in vivo* studies on SR and supragingival DPB. It was emphasized that rough surfaces cause both accumulation and retention of more DPB. After several days of undisturbed DPB formation, a more mature plaque with a higher proportion of motile organisms and spirochetes can be observed on rough surfaces. Also, crowns with rough surfaces are

more likely to be surrounded by an inflamed periodontium (higher bleeding index, increased production of crevicular fluid and/or inflammation observed histologically).

A threshold value for SR was first put forward by Quirynen et al. (57) on 1996. This study was designed to examine the effect of surface polishing on supragingival and subgingival bacterial colonization in the short-term. Four different titanium abutments with different SRs ranging from highly polished to standard surfaces were compared within the same subject. Both quantitative and qualitative microbiologic examinations and clinical examinations were made. It was concluded that below a threshold value of SR no major changes were observed in microbiological composition, bacterial adhesion and/or colonization. The same study also concluded that the abutment with a Ra value of 0.21μm provided an attachment gain of 0.2mm during the 3 months whereas a loss of attachment was observed around abutments with a lower Ra. It was stated that a certain SR is necessary for increasing resistance to clinical probing.

Bollen et al. (51) (1996) evaluated the long-term effects of smoothening hard transgingival surfaces. A standard machined titanium abutment (Ra: 0.21μm) was compared with a ceramic material (Ra: 0.06μm). Evaluations were made after 3 months of oral exposure and after 12 months. It was observed that the spirochetes and motile organisms harbored by both abutment types were proportioned equally both supra- and subgingivally. Large inter-abutment differences were not detected by microbial culturing even though there were some slight variations. The differences in CFU for both aerobic and anaerobic bacteria were within one division of a logarithmic scale. The smoothest abutments resulted in a slightly higher increase in PPD between 3 months and 12 months. Also, more BoP was observed in the smoothest abutments. This study confirmed the findings of the short-term study of Quirynen et al. (57). It was concluded that below a "threshold Ra" (0.2 μm) the supra- and subgingival microbial composition were not affected.

A review conducted in 1997 by Bollen et al. (173) also evaluated initial SR of different dental materials including teeth, resin composite and ceramics; and how different treatment modalities such as polishing, scaling and glazing affected the initial

SR. The effects were also analyzed and compared to the threshold SR of 0.2 μm. This review concluded that the SR range of intra-oral hard surfaces was wide and the effect of different dental treatment modalities on SR was dependent on the material. It was also stated that "every dental material" needed "its own treatment modality in order to obtain and maintain a surface as smooth as possible".

2.5.1. Surface Roughness of Teeth

The SR of hard surfaces present in the oral cavity can have an influence on bacterial adhesion (173). The natural hard surfaces present in the oral cavity are teeth. It has been shown that the presence of gingivitis or periodontitis is more often associated to teeth with rough and contaminated surfaces (172). Majority of the studies conducted to evaluate SR of teeth concluded that periodontal treatment and oral hygiene practices (174) may result in various changes of the tooth surface (60,127,154,174,175).

2.5.2. Surface Roughness of Ceramics

Prominence has been given to the use of ceramic restorations over the years (176) due to an increase in patients' esthetics demands, improvements of the mechanical and esthetic properties of the materials, minimally invasive preparation options and fabrication methods available (177).

Ceramics are safe to use for restorations in the oral cavity since they are stable, biocompatible and inert materials (177). There are 2 types of ceramic restorations where ceramics are used in the field of prosthodontic dentistry. These are porcelain fused to metal restorations and all-ceramic restorations. Main disadvantages of porcelain fused to metal restorations are their dark appearance in color (50) especially at the cervical area and the possibility of allergic reactions in some patients due to the presence of metal elements in the metal alloy. All-ceramic restorations on the other hand are made completely out of ceramic materials providing better esthetics and marginal adjustment. They can be monolithic or bi-layered (having a metal core covered with a ceramic veneer) (178,179). The main disadvantage of bi-layered all-ceramic restorations is their

susceptibility to delamination and fracture (180). Monolithic restorations are more durable compared to bi-layered restorations (181). Also, with monolithic restorations, a proper occlusal morphology and proper occlusal contacts can be achieved through pressing or computer-aided design and computer-aided manufacturing (177).

Due to their biocompatibility, esthetics and strength; all ceramic dental materials are seen as the first choice of material for many different dental applications. Their appearance visually simulates the characteristics of teeth successfully (182). Currently there are many systems and a wide range of ceramic materials available on the market (179,183).

The final surface finish of ceramic restorations which is considered to be ideal is obtained mainly by glazing (184-187) to achieve a smooth restoration surface as a laboratory procedure. For glazing a restoration, the finished restorations are coated with an esthetic and hygienic glass (183). Glazing produces a restoration surface with a light reflection similar to natural teeth and discourages plaque accumulation on the surface (188). If the glazed surface is broken, the ceramic surface is polished and/or re-glazed to achieve the best surface finish possible (189). If a correction is to be made to a glazed porcelain resulting in a rough and ground porcelain surface, it may induce biological and physical problems including increased tendency for plaque accumulation (54,187,190), inflammatory reactions on the mucosa (191) and a harmful influence on periodontal tissues (53). Furthermore, it has been shown that periodontal instrumentation, which is required to maintain periodontal health around restorations, is also a factor which affects the SR of ceramic restorations (192,193).

The study of Kawai et al. (53) (2000) examined how the SR of porcelain disks affected the adhesion of both bacterial cells and glucans. It was concluded by the authors that since glazing may produce an "undulating rough surface that usually has irregularities" due to lack of technical sensitivity during manufacturing further polishing may be beneficial from a biological point of view. The study of Brentel et al. (187) (2011) found a positive correlation between SR of the ceramic surfaces and the biovolume of plaque accumulated on the surface and a positive correlation between SR of the ceramic

surface and mean thickness of biofilm. The study of Al-Marzok et al. (194) (2013) also found a positive correlation between the SR of ceramic and *streptococcus* mutans adhesion.

Gathered findings from the literature confirm that a generalization cannot be made on how a smoother ceramic surface can be obtained (185,186,189,191,195,196). Since new ceramic materials and new polishing systems are being introduced, there is a need for further research. Clarification is expected about which polishing system produces the smoothest finish in which type of ceramic through the ongoing and future debate A clinician should understand that there are specific requirements for fabricating and polishing different types ceramic materials (189).

2.5.3. Surface Roughness of Resin Cements

After cementation of restorations, removing the excess cement is challenging since it is very difficult to access the approximal surfaces, gingival crevices and the complex root topography at the restoration interface (197). There are different methods used to remove the excess luting material. Two of the most popular methods are “wiping with a cotton pellet directly after cementation and flicking off with a hand instrument or scalpel after initial polymerization of the luting cement.” (198). It has been shown that subclinical cement retention occurs after cementation even after the removal of all excess cement as judged by the naked eye (197) and this excessive cement present at the tooth restoration interface results in an increase of SR (198). As a result inflammation may develop due to plaque accumulation and also bone resorption as well as root surface demineralization may take place (198).

Anami et al. (199) (2012) studied how different excessive cement removal techniques affected the SR and bacterial adhesion at the restoration interface. They have concluded that the Ra values ranged from 0.96 to 1.69 μm after different techniques were applied. The Ra values obtained from the adhesive interface were above the threshold SR value. Through the evaluation of biofilm adhesion at the adhesive interface, it was concluded that the interface SR and morphology were associated with bacterial

accumulation.

In a similar study design Pereira et al. (200) (2016) evaluated how polishing subsequent to the removal of excessive cement using different techniques affected the surface topography and bacterial adhesion. When the interface was not polished, the SR values ranged from Ra: 2.7 to 4.6 µm. When polished, smoother surfaces were obtained, however SR values still ranged from Ra:1.7 to 2.5 µm. All these values are above the threshold SR values. It was concluded that marginal polishing had a pivotal role in achieving smoother surfaces. The findings of Anami et al. (199) were confirmed by this study since it was observed that the excess cement around the edges may increase SR and influence bacterial adhesion.

Currently there is paucity of information on how polishing effects the cement removal methods or restorative margins, SR and its effects on bacterial colonization and further studies in this area are required (200) .

2.6. The Relationship Between Restoration Margins and Periodontal Health

The effect of restoration margins on the periodontal health of the patients has been a very important research topic in the field of periodontology. As a demand for restorations increases, the importance of periodontal treatment around restoration margins increases as well. It has been shown that the placement of a dental restoration margin may cause gingival inflammation (64,67-71). Yet it was also claimed that through self-performed oral hygiene practices and regular SPC instrumentation after prosthodontic therapy, periodontal health can be maintained with fixed prosthodontic restorations (201,202).

Even though it was found that supragingival position of the crown margins is the most favorable location for periodontal health (203), subgingival placement of margins may be indicated in certain situations. Esthetic concerns, removal of previous restorations or dental caries, crown fractures, abfraction, abrasion, chemical erosion, susceptibility to root caries, enhancement of retention and resistance form, development of a ferrule effect

when post-core is used may oblige the clinician to prefer a subgingival margin placement (68,204-208).

There is a higher incidence of gingival inflammation around clinically deficient restorations, restorations with poor marginal adaptation, restorations with rough surfaces and restorations with deep subgingival finish lines. However, it has also been shown that gingival inflammation may develop around polished and well contoured restorations (66).

Newcomb (68) (1974) studied the relationship between the location of the subgingival crown margins and gingival inflammation. It was concluded that deep subgingival margins were positively correlated with increased gingival inflammation. It was also stated that the plaque indices of crowned teeth were found to be lower compared to uncrowned teeth since plaque bacteria retained in the niche between the restoration and the teeth where they would not be accesses to be measured by the PI. This study also stated that, in cases where a subgingival margin placement is unavoidable, the minimum achievable extension into the gingival sulcus should be aimed for.

Valderhaug and Birkeland (71) (1976) studied the periodontal conditions of 114 patients following the insertion of fixed prosthesis for a period of 5 years. Oral hygiene, gingival condition, PPD and loss of attachment were evaluated. It was observed that the percentage of subgingival margins were %65 at the baseline and dropped to %41, 5 years later. Higher gingival index (GI) scores, deeper PPDs and an increase in loss of attachment with recession were seen in margins placed subgingivally.

Lang et al. (67) (1983) compared the clinical and microbiological effects of subgingival restorations with overhanging versus clinically perfect margins. It was observed that the subgingival flora around the overhanging margin was similar to the subgingival flora observed in chronic periodontitis patients. Around clinically perfect margins, a microflora of initial gingivitis or gingival health was observed.

Schatzle et al. (70) (2001) investigated the long-term relationship between subgingival dental restorations and periodontal health over a period of 26 years (from

1969 to 1995). The participants were middle and upper middleclass Scandinavian males with good to moderate oral hygiene. Throughout the 26-year period, inflammation around subgingival restorations (98 tooth surfaces with subgingival fillings extending to more than 1mm below the gingival margin) was always noticeably more compared to the inflammation observed around the gingival border of the control group (615 sound/supragingivally restored tooth surfaces). A loss of attachment was observed in the test group immediately following the placement of subgingival restorations. Over the next 8 years the attachment level was stable. Afterwards another significant increase in the loss of attachment was observed. This second increase was associated with an increase in gingival caries index as well. It was also seen that some subgingival restorations became supragingival over time due to recessions. In this study, it was concluded that the restorations placed below the gingival margin were harmful to both gingival and periodontal health. It was also observed that an increased loss of attachment started slowly, 1-3 years were required for clinical detection of the loss after the placement of the restorations.

Al-Wahadni et al. (64) (2006) evaluated the periodontal response to all-ceramic crowns in general practice. Eighty-two crowns were placed in 64 patients. After an average of 16.27 months, periodontal health status was recorded (plaque index (PI), GI and PPD). Same measurements were taken from the matching contralateral teeth by one calibrated examiner. The PI, GI and mean PPD of crowned teeth presented less favorably to the scores of the control teeth. The study concluded that teeth with monolithic all ceramic IPS Empress crowns had more clinically evident plaque compared to uncrowned teeth. The study also highlighted the importance of oral hygiene knowledge, oral hygiene skills and self-care behaviors of patients who have or who will receive all ceramic crowns.

Paniz et al. (69) (2016) evaluated the periodontal effects of the presence of a single restoration with a subgingival margin in terms of soft tissue parameters in the anterior maxilla. They also compared two different preparations (chamfer n=106 vs. feather edge n=94). PI, GI, PPD, BOP and phenotype of the soft tissue were recorded. Twelve months after the delivery of the restorations, same parameters were re-evalauted. Compared to the baseline, a significant increase in PI, GI, BoP and PPD was measured. There was not

a statistically significant difference between different finishing lines regarding these parameters. Feather edge preparation had a statistically significantly higher incidence of BOP and significantly less gingival recession compared to chamfer preparation. This study concluded that subgingival margins influenced the inflammatory soft tissue response.

The narrative review of Ercoli and Caton (65) (2018) concluded that “tooth supported and/or tooth-retained restorations and their design, fabrication, delivery and materials have often been associated with plaque retention and loss of attachment”. In the same review it is also emphasized that “if patients are compliant with self-performed plaque control and periodic maintenance”, restoration margins within the gingival sulcus may not cause gingivitis. However, it is also stated that restoration margins placed within the junctional epithelium and supracrestal connective tissue attachment are associated with inflammation and recession.

3. MATERIALS AND METHODS

The present study was approved by the Scientific Committee of Yeditepe University (Scientific Committee no:345). Preparation of dentin specimens (DS) from bovine teeth, cementation of DSs to reciprocating ceramic specimens (CS) and profilometric analysis was conducted in Yeditepe University, Faculty of Dentistry, hard tissue lab. CSs were prepared in a private dental prosthetics laboratory. Scanning electron microscope (SEM) evaluation was done in TUBİTAK Marmara Research Center Materials Institute.

3.1. Sample Number Calculation

Prior to the study, a power analysis was performed: α:0.05, Power (β) of 80%, d effect size of 0.84 for the Ra parameter and standard deviation (SD) of:0.5. Calculated sample number was found to be minimum n=18 for each group using the research paper Schlageter et al. (175). G*Power program was used for the power analysis. In this in vitro study, the sample size was designated as n=20 to compensate for any loss of specimens during the experiments.

3.2. Sample Preparation

3.2.1. Preparation of DSs

All steps of DS preparation are summarized in Figure 3.1.

Permanent lower anterior bovine teeth (n=100) of freshly slaughtered cattle from the abattoir of Keşan Municipality, were extracted. Slaughtered cattle were among approximately 3 years old animals for standardization. Central incisors (first pair) were used in the study. For the prevention of bacterial growth, teeth were immediately placed in deionized water containing thymol and were kept at 4°C until the day of specimen preparation. Specimens were prepared within 2-3 months.

After being removed from deionized water, soft tissue residues were cleaned by using periodontal curettes and polishing with pumice and brushes (Figure 8).

Crowns of the teeth were removed with a transverse cut below the cementoenamel junction by flame coarse diamond burrs[3] using constant air-water cooling with a high-speed dental handpiece. A second transverse cut parallel to the first one was made approximately 12 mm apical to the first cut (Figure 9). Of the two obtained root specimens, the coronal piece was used for the experiment (Figure 10). The buccal site of the coronal root section was first marked with a permanent marker.

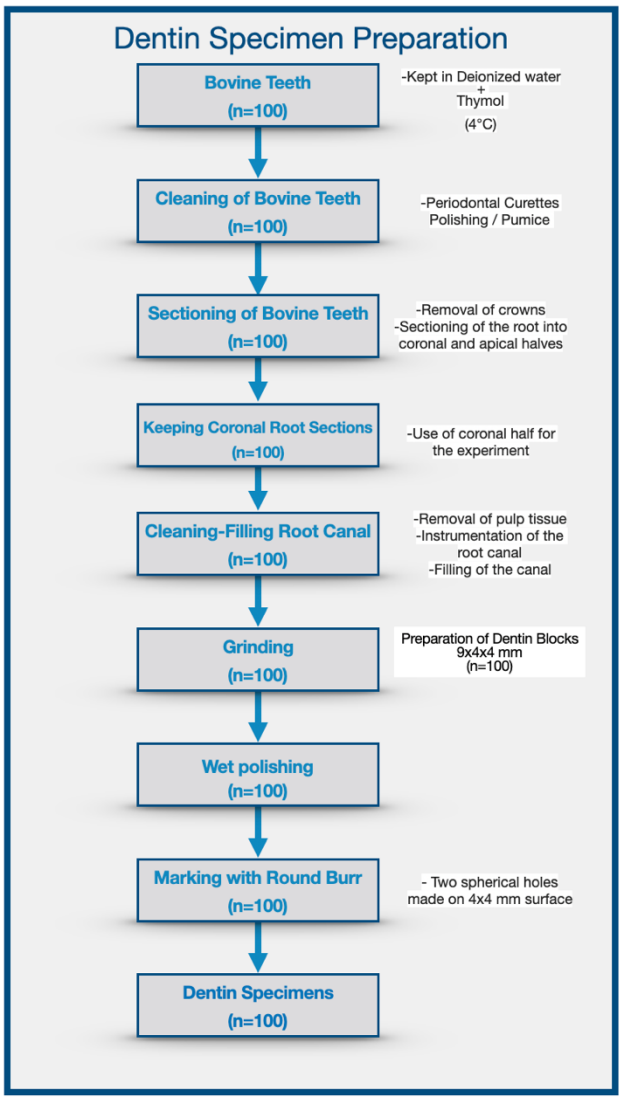


Figure 3.1. DS Preparation

[3] **Diamond Coarse Round Burr**, Acurata, Thurmansbang, Germany



Figure 3.2.
Bovine incisor

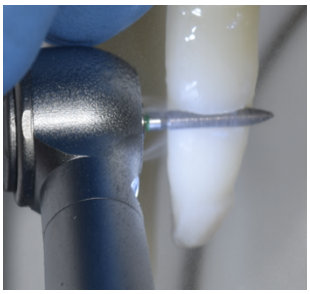


Figure 3.3.
Sectioning of root

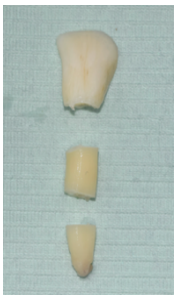


Figure 3.4.
Sectioned bovine incisor

The pulp tissue present in this root section was removed by using a size 80, stainless steel K-File[4] (Figure 3.5). A flame coarse diamond burr with a high-speed dental handpiece was used to smoothen the inner wall of the canal and remove any left-over soft tissue residue under constant air-water cooling (Figure 3.6).

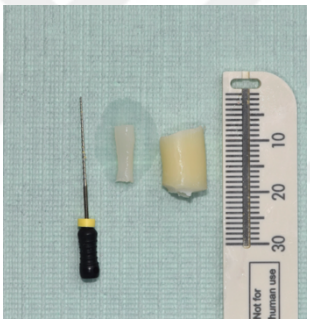


Figure 3.5.
Coronal root section



Figure 3.6.
Removal of residual pulp tissue

To turn the root section into a firm intact block and enable easy handling of the specimen, the apical opening of root canal was obstructed with resin composite[5] (Figure 3.7 – 3.8) and then filled with flowable restorative material[6] (Figure 3.9).

[4] **K-File**, Dentisply, Brea, CA, USA
[5] **SOLARE X**, GC, Bunkyo-ku, Tokyo, Japan
[6] **Aelite Flo**, Bisco, Anaheim, CA, USA



Figure 3.7. Restorative material



Figure 3.8. Obstructed root section

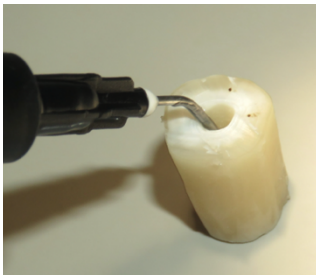


Figure 3.9. Filling with flowable restorative material

After the application of restorative materials, each specimen was shaped into a rectangular prism. On all four surfaces as buccal, mesial, distal and palatal sides, specimens were grinded and polished with silicon carbide papers adjusted to a polishing device[7]. Both grinding and polishing procedures were done one by one parallel to the long axis of the root section with #180, #220, #600, #1000, #1500, #2000 grit silicon carbide papers[8] under constant water cooling. The buccal site was re-marked with a permanent marker after the polishing procedure since the previous marking was lost during this stage. The top and bottom were also grinded and polished parallel to the horizontal axis (following #180 to #2000 grit numbers). At the end of the steps above, coronal root sections, are now shaped as rectangular prisms with 9x4x4 mm dimensions as DSs. Since reciprocating CSs will be custom-made for each DS, small guiding holes created with a 2mm round coarse diamond burr on the coronal plane surface of each DS were needed to achieve proper positioning of the CSs-DSs junction. The depth and insertion axis of the burrs were standardized.

DSs were kept in deionized water containing thymol at 4°C in order to prevent bacterial growth till the (computer aided design and computer aided manufacturing) CAD/CAM scanning procedure.

[7] **MetaServ**® 250, Buehler, Lake Bluff, IL, USA
[8] **Wetordry**™, 3M, St. Paul., Minnesota, USA

3.2.2. Preparation of Ceramic Specimens

All the steps of CS preparation are summarized in Figure 3.10.

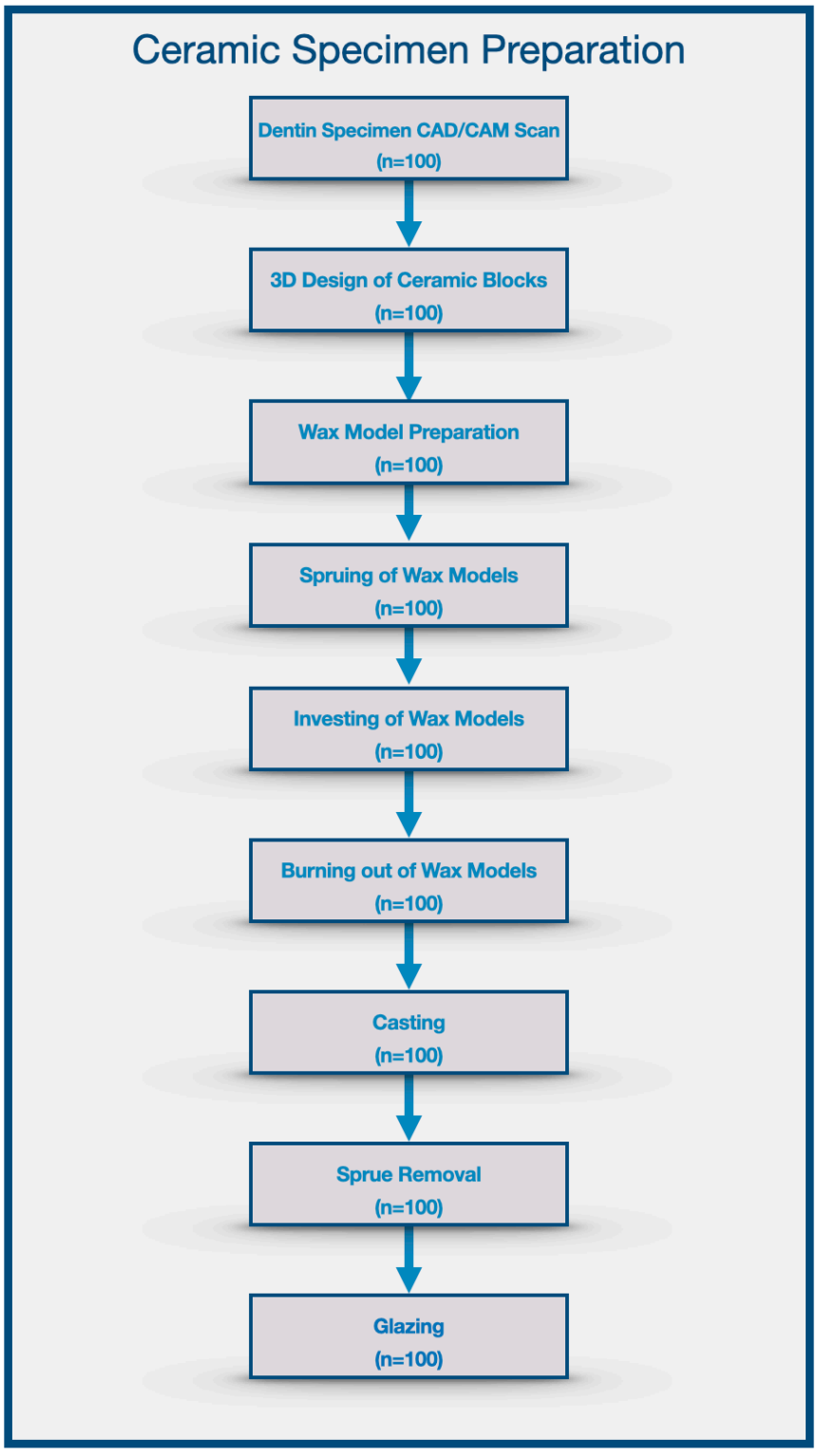


Figure 3.10. CS preparation

CSs were obtained with the lost-wax technique. CSs were prepared to individually fit each DS. In the first step, DSs were sprayed using white CAD/CAM scanning spray[9] (Figure 3.11) to enable CAD/CAM scanning with a lab scanner[10]. 3D images of DSs were transferred to the software and for each DS, a CS with a perfect fit was designed (Figure 3.12). Once the design was completed, a milling wax[11] was placed in the dental milling machine[12] to produce a wax model (Figure 3.13-3.15).

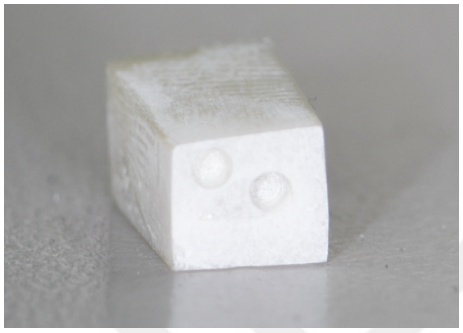


Figure 3.11.
Sprayed DS

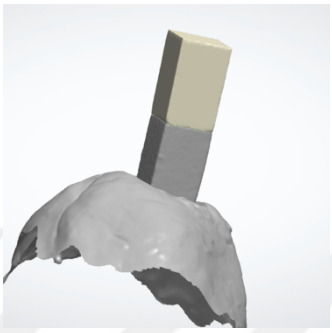


Figure 3.12.
3D scan of the DS and individual CS design

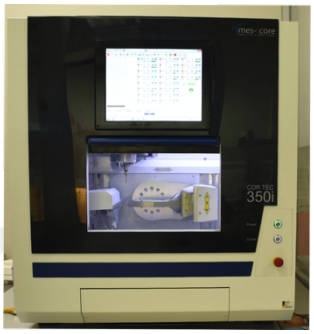


Figure 3.13.
Dental milling machine

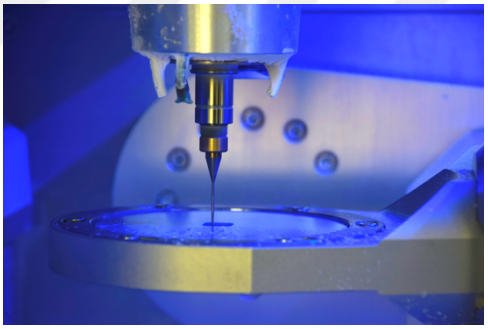


Figure 3.14. Milling of the wax



Figure 3.15.
Finished wax model

A wax sprue was placed onto the wax model immediately after milling to prevent any sort of distortion (Figure 3.16). The free end of the sprue was attached to the apex of the sprue base (Figure 3.17).

[9] **Dr. Mat**, Mat Kimya, İstanbul, Turkey
[10] **E1**, 3 Shape A/S, Copenhagen, Denmark
[11] **ProART CAD Wax blue**, Ivoclar Vivadent AG, Schaan, Liechtenstein
[12] **Coritcec 350i**, Imes-icore, GmbH, Eiterfeld, Germany

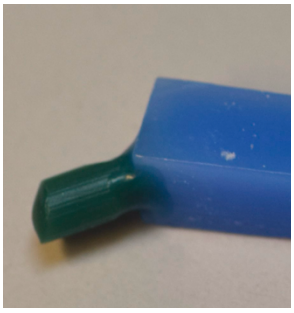


Figure 3.16.
Wax sprue attached to the wax model

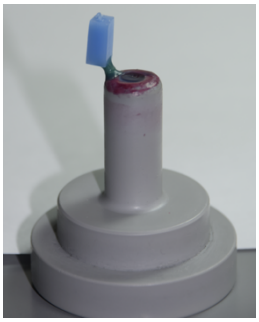


Figure 3.17.
Sprue attached to the sprue base

A silicone ring[13] was placed onto the sprue base (Figure 3.18). It was assured that the silicone ring fitted the base to prevent any leakage of the investment material (Figure 3.19). A universally suitable investment material[14] was prepared in a power-driven vacuum mixer. A brush was used to apply some investment material to the small indentations of the wax model. It was then poured slowly to the empty space within the silicon ring until the desired level was reached (Figure 3.20). The ring gauge[15] was placed with a hinged movement. At this stage, the preparation of the investment ring was completed, and it was left untouched to set. After being set, the investment ring was removed from the silicone ring and the ring base (Figure 3.21).

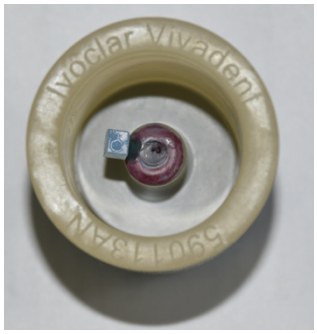


Figure 3.18.
Silicone ring



Figure 3.19.
Investment material



Figure 3.20.
Investment material being poured



Figure 3.21.
Investment ring

[13] **IPS Silicone Ring,** Ivoclar Vivadent, Amherst, NY, USA
[14] **IPS® Press Vest Premium**, Ivoclar Vivadent, Amherst, NY, USA
[15] **IPS Ring Gauge**, Ivoclar Vivadent, Amherst, NY, USA

The investment ring was placed inside the pre-drying furnace with the opening face down (Figure 3.22). During this burnout step, the wax model was evaporated from the investment ring and a mold was created. After the completion of this step, both the cold ingot[16] and the cold plungers[17-18] were placed into the investment ring facing up. This loaded investment ring was then placed inside the preheated press furnace (Figure 3.23) and instructions of the manufacturer were followed during the pressing program. Once the program was over, the investment ring was removed immediately from the furnace and was left to cool down without any interference to accelerate the cooling process (Figure 3.24).



Figure 3.22.
Investment ring inside the pre-drying furnace

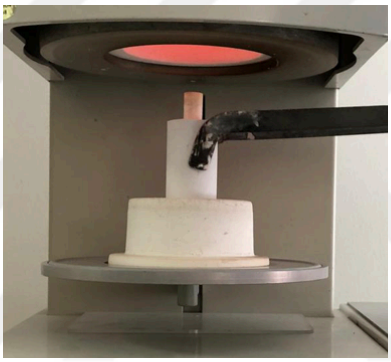


Figure 3.23.
Investment ring being placed inside the press furnace



Figure 3.24.
Investment ring cooling down

After cooling down (Figure 3.25), the investment ring was separated with a separation ring (Figure 3.26-3.28). To divest the ceramic blocks polishing beads were used. During the rough divestment stage (Figure 3.29), 4 bar (58 psi) pressure was used whereas 2 bar (29psi) for the fine divestment stage. All these stages were carried out by paying maximum attention to the ceramic blocks to prevent any possible damage. A suitable separation disc at low speed was used to remove the sprue (Figure 3.30).

[16] **IPS e.max Press MT BL4,** Ivoclar Vivadent, Amherst, NY, USA
[17] **IPS Multi One-Way Plunger,** Ivoclar Vivadent, Amherst, NY, USA
[18] **IPS Alox Plunger,** Ivoclar Vivadent, Amherst, NY, USA

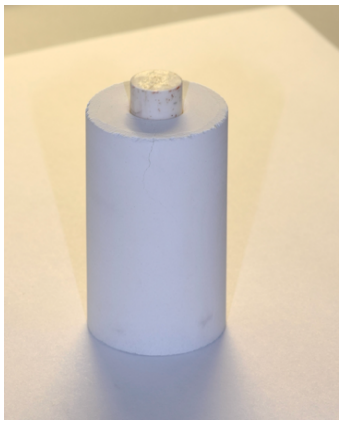


Figure 3.25.
Cooled down investment ring

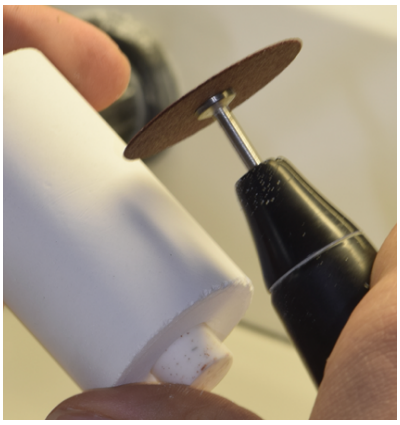


Figure 3.26.
Marking of the investment
.



Figure 3.27.
Seperation of the investment



Figure 3.28
Seperated blocks containing CS within investment material



Figure 3.29.
Rough divestment

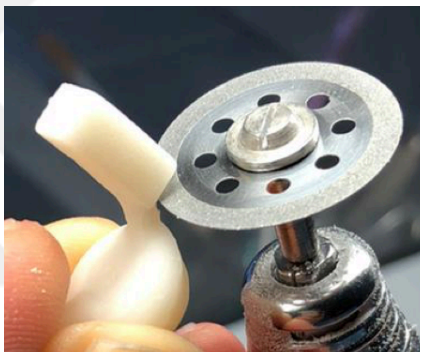


Figure 3.30.
Sprue removal

In order to glaze the surface, glaze powder[19]and mixing liquid[20] (Figure 3.31) mixed with a glass stick (Figure 3.32) were used.

[19] **IPS Ivocolor Glaze Powder**, Ivoclar Vivadent, Amherst, NY, USA
[20] **IPS Mixing Liquid Allround**, Ivoclar Vivadent, Amherst, NY, USA



Figure 3.31.
Glaze powder and mixing liquid

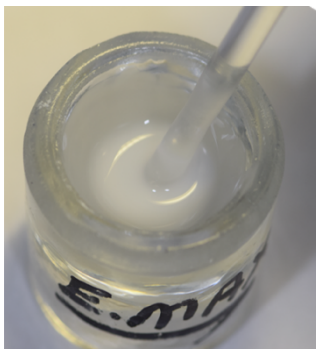


Figure 3.32.
Prepared glaze

The glaze was applied with a brush as an even layer to cover the surfaces of CS (Figure 3.33). Afterwards, the CS was placed on a honey-comb firing tray. Firing parameters recommended by the manufacturer were followed (Figure 3.34). Once the firing was over, the CS was left to cool down without any interference to accelerate the cooling process (Figure 3.35).



Figure 3.33.
Application of glaze

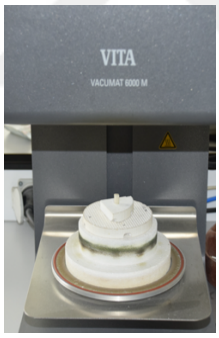


Figure 3.34.
Glaze firing

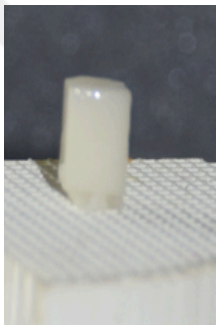


Figure 3.35.
Cooling down

All DSs and CSs were placed inside an ultrasonic cleaner containing distilled water for 5 minutes (Figure 3.36-3.37).



Figure 3.36.
Ultrasonic cleaner



Figure 3.37.
DSs and CSs inside the ultrasonic cleaner

3.3. Cementation of DSs to CSs

Cementation steps are shown in Figure 3.38.

The surfaces of DSs which were to be cemented were washed with water spray and air dried. Thereafter they were treated with an etching gel containing %37 phosphoric acid[21] for 15 Seconds (Figure 3.39-3.40) followed by washing with the water spray and air drying (Figure 3.41). The adhesive[22] (Figure 3.42) was applied to the surface for a duration of 20 seconds with a microbrush (Figure 3.43) and diffused with air to prevent pooling (Figure 3.44). The adhesive material was then cured with the LED Curing Light[23] for 10 seconds (Figure 3.45). DS and CS surfaces were prepared simultaneously. The surfaces of CSs facing the DSs were washed with water syringe and air dried. CSs were pre-treated with hydrofluoric acid[24] for 60 seconds (Figure 3.46-3.47). The acid was rinsed with water spray and air dried (Figure 3.48). The ceramic surface was primed[25] by a microbrush for a duration of 60 seconds (Figure 3.49-3.50). After 60 seconds, the material was diffused with an air spray (Figure 3.51).

[21] **N-Etch,** Ivoclar Vivadent, Amherst, NY, USA
[22] **Adhese Universal**, Ivoclar Vivadent, Amherst, NY, USA
[23] **D-Light® Pro,** GC, Bunkyo-ku, Tokyo, Japan
[24] **IPS Ceramic etching gel,** Ivoclar Vivadent, Amherst, NY, USA
[25] **Monobond Plus®,** Ivoclar Vivadent, Amherst, NY, USA

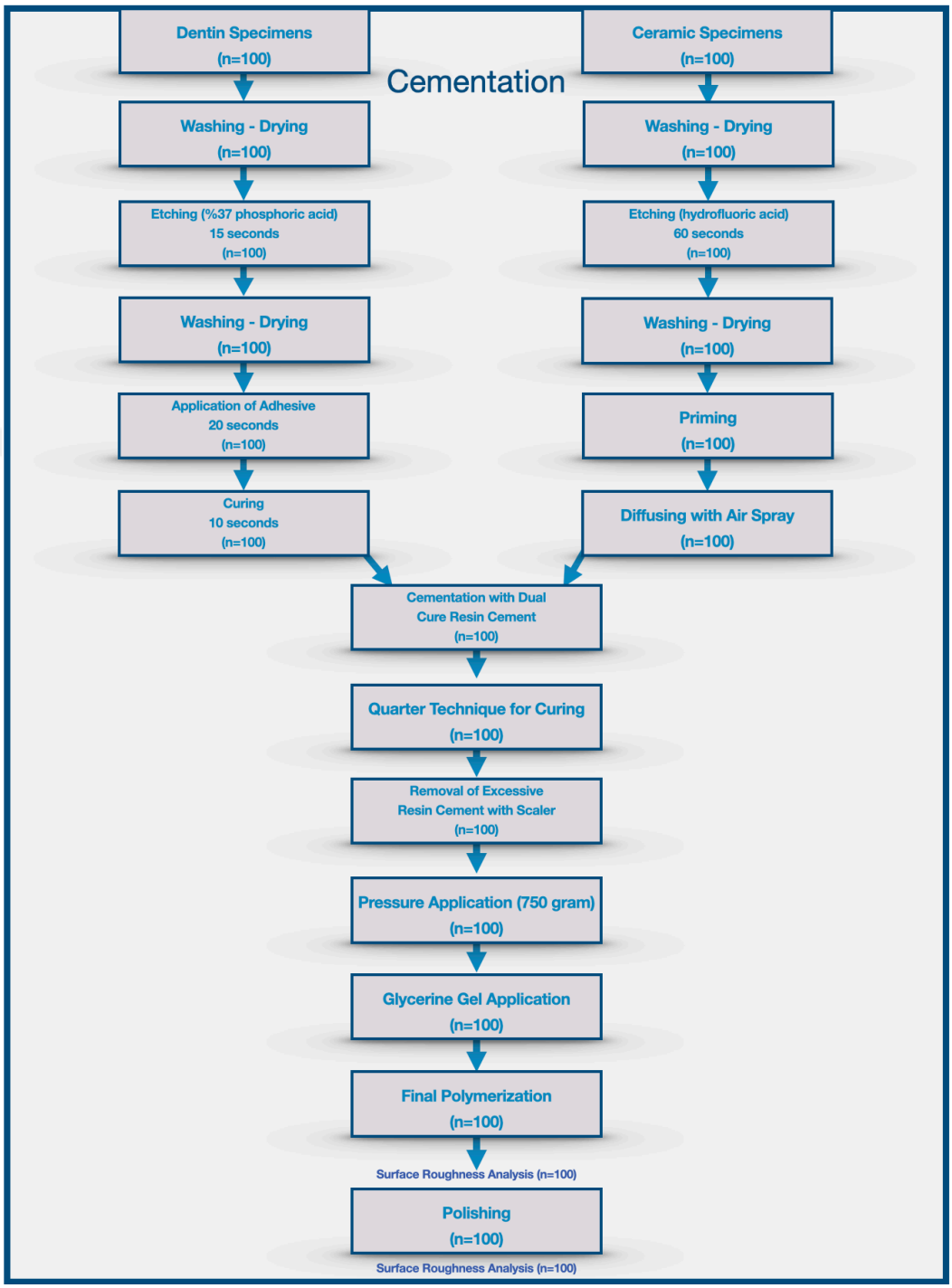


Figure 3.38. Steps of cementation

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Figure 3.39.

Etching gel

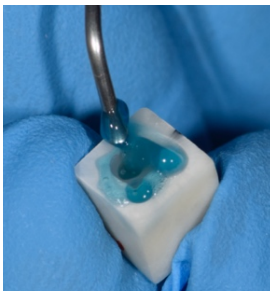


Figure 3.40.

Etching gel application

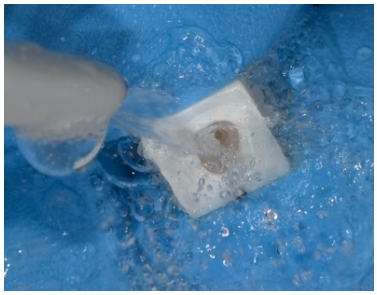


Figure 3.41.

Washing with water spray



Figure 3.42.

Adhesive

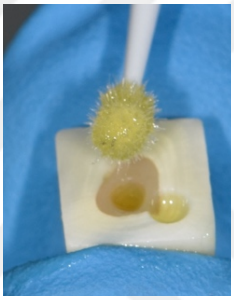


Figure 3.43.

Adhesive application

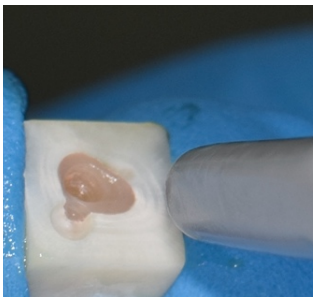


Figure 3.44.

Diffusing the adhesive with air spray

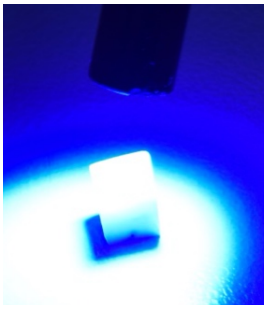


Figure 3.45

Curing



Figure 3.46. Hydrofluoric

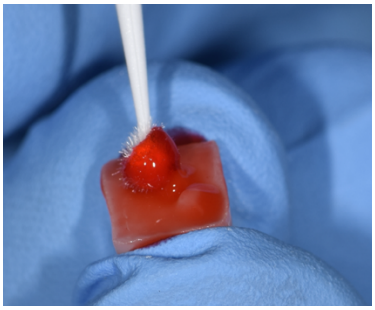


Figure 3.47. Hydroflouric acid application



Figure 3.48. Washing with water spray



Figure 3.49. Primer

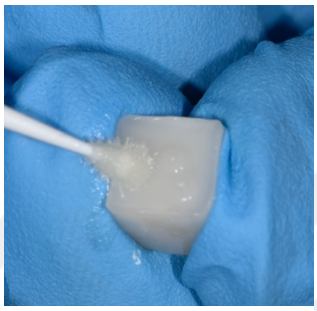


Figure 3.50. Primer application



Figure 3.51. Diffusion of the primer with air spray

Once surface preparations were completed, reciprocating CSs were cemented to DSs with a dual cure resin cement[26] (Figure 3.52). The quarter curing technique (2 seconds for each surface at a distance of 9 mm) was used before removing the excess material. The excess material was removed quickly with a sickle scaler (Figure 3.53). Pressure was applied to the cemented block for a duration of 1 minute.

[26] **Variolink Esthetic DC,** Ivoclar Vivadent, Amherst, NY, USA)



Figure 3.52.
Dual cure resin cement

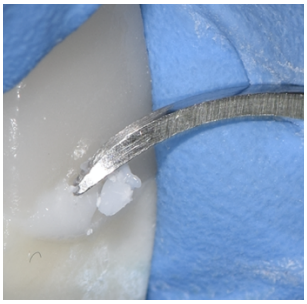


Figure 3.53.
Removal of excessive cement

In order to prevent the formation of oxygen inhibition layer on the interface, immediately after the removal of excess cement material, DS-CS junction were covered with glycerine gel[27] (Figure 3.54-3.55) The glycerine gel was rinsed off after final polymerization (Figure 3.56). Specimens were kept immersed in 37°C for 24 hours after cementation.



Figure 3.54
Glycerine gel

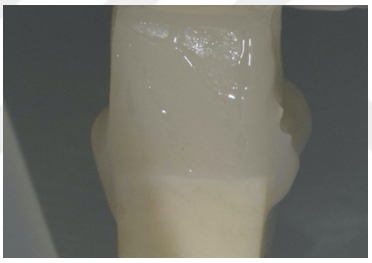


Figure 3.55.
After glycerine gel application

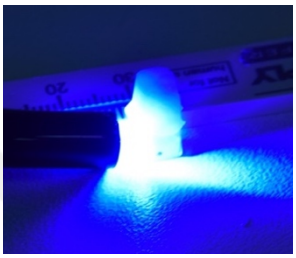


Figure 3.56.
Final polymerization

3.4. Polishing of the Cemented Blocks of DS-CS

After the cementation was completed all blocks were polished by a universal polisher[28]. The rotation speed was set at 9000 rpm under water spray during polishing (Figure 3.57) with circular movements. The entire buccal block surface was polished for 60 seconds (Figure 3.58).

[27] **Liquid Strip**, Ivoclar Vivadent, Amherst, NY, USA
[28] **Optragloss**, Ivoclar Vivadent, Amherst, NY, USA

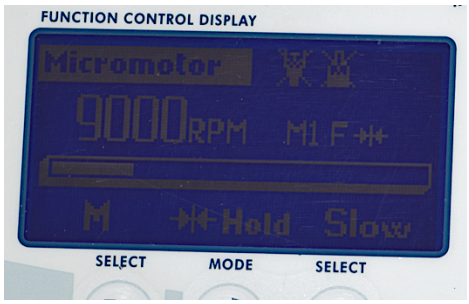


Figure 3.57.
Rotation speed during polishing



Figure 3.58.
Polishing

3.5. Allocation of the Cemented Blocks into Study Groups

Each block was coded and the code number (1-100) was written at the base of the dentin side. Blocks were randomly divided into 5 groups for the application of different periodontal tools through mechanical instrumentation (Figure 3.59). A randomization website was used (randomlists.com) to assign the blocks into groups (Figure 3.60) and the group numbers were also marked with roman numerals at the base (Table 3.1).

-Group I: Titanium nitrite coated curette (TiN) instrumentation (TiN Group).
-Group II: Ultrasonic scaler application (US Group).
-Group III: Linear oscillating device application together with a hydroxyapatite-based polishing fluid (LOD Group)
-Group IV: Supragingival AP with the supra nozzle and a trehalose APP with ≈65µm grain size (coarse APP) (AP-S Group).
-Group V: Subgingival AP with the perio nozzle and a trehalose APP with ≈30µm grain size (fine APP) (AP-P Group).

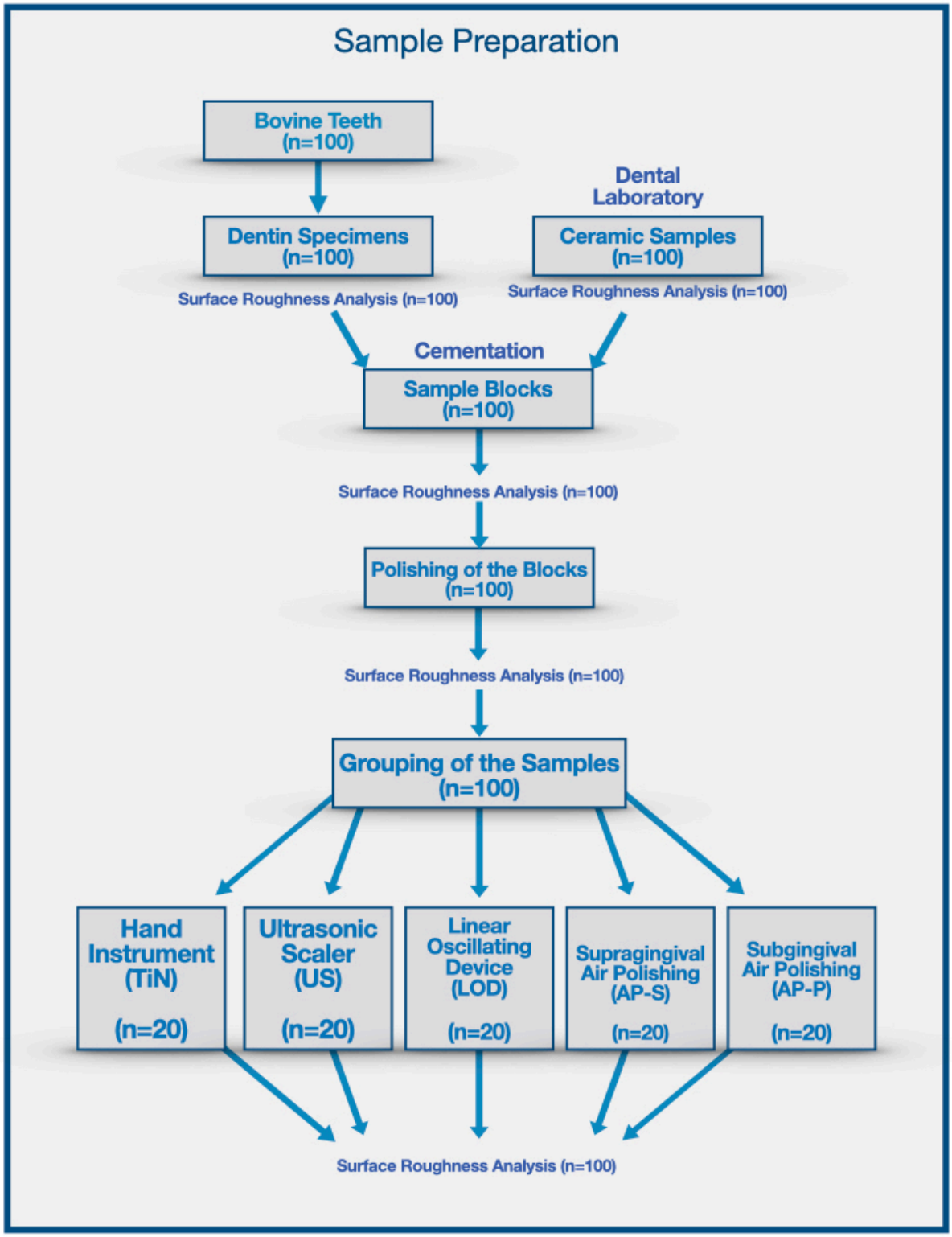


Figure 3.59. Flowchart of the experimental period

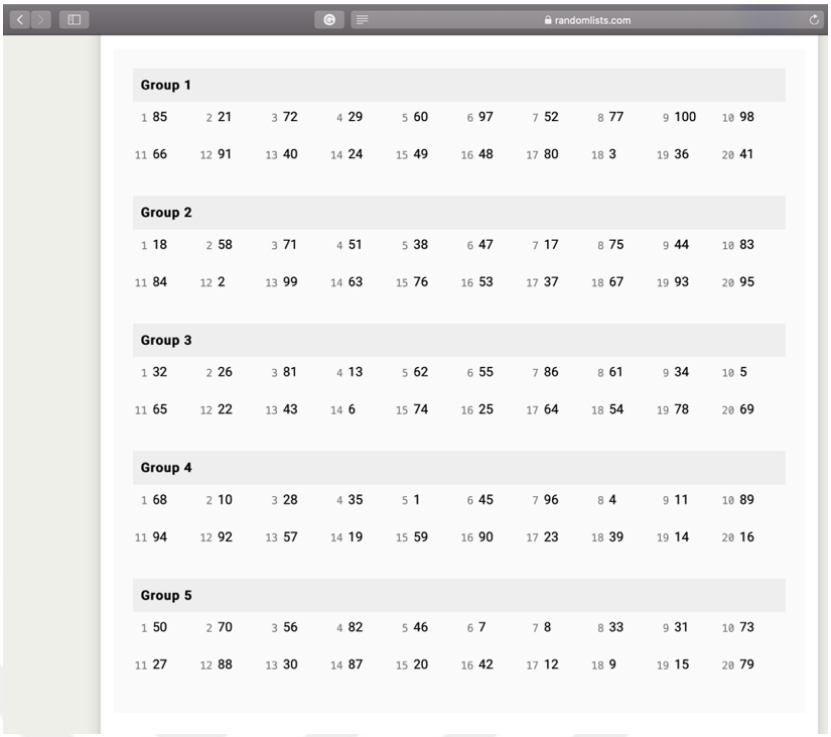


Figure 3.60. Randomization table

Table 3.1. Distribution of blocks to experimental groups

Group	Sample Size (n)	Procedure
I	**20**	Instrumentation with TiN curette
II	**20**	US application
III	**20**	LOD application
IV	**20**	AP with coarse APP *(supra nozzle / grain size: ≈ 65 µm)*
V	**20**	AP with fine APP *(perio nozzle with perio tip / grain size: ≈ 30 µm)*

3.6. Instrumentation with Stable Lateral Forces

To perform with standardized stable lateral forces, the operator performing all surface treatments (DFB) was previously trained on extracted teeth as explained by Kawashima et al. (209) using the method of Kishida et al. (144). The pressure for ultrasonic instruments (US, LOD) was aimed to be 40 grams (0,39 Newton) whereas pressure of hand instrumentation (TiN curette) 500 grams (4,9 Newton) (144,210).

The operator treated a tooth surface for 3 methods as TiN curette, US and LOD for a total of 200 seconds each. At intervals of 10 seconds the applied force was measured with a force gauge (32) (Figure 3.61). This training work showed that DFB applied a lateral force of 485,5±8,87 grams during TiN curette instrumentation, 38±6,959 grams during US application and 38±5,231 grams during LOD application.

The intraclass correlation coefficient was calculated by repeating the instrumentation on the force gauge for each instrument and was found to be 0,921(%95 confidence interval (CI)), 0,878 (%95 CI) and 0,926 (%95 CI) for TiN, US and LOD, respectively.



Figure 3.61. Instrumentation on the force gauge

3.7. Titanium Nitrite Coated Curette Instrumentation

Twenty specimens were instrumented with the TiN curette[29] (Figure 3.62-3.64). The curette was held parallel to the long axis of the block. The terminal shank was also held parallel to the surface of the block. The curette was adapted by placing the lower cutting edge against the surface. Lateral pressure was applied to the block during the upward pull. Representing apicocoronal movements, strokes started from the base of the DS towards the CS following the long axis of each block. Imbricate movements were used, and surface of the block was traced. The application was performed for a duration of 60 seconds. The number of strokes in each application was counted to be approximately 20.



Figure 3.62. TiN curette



Figure 3.63. TiN curette

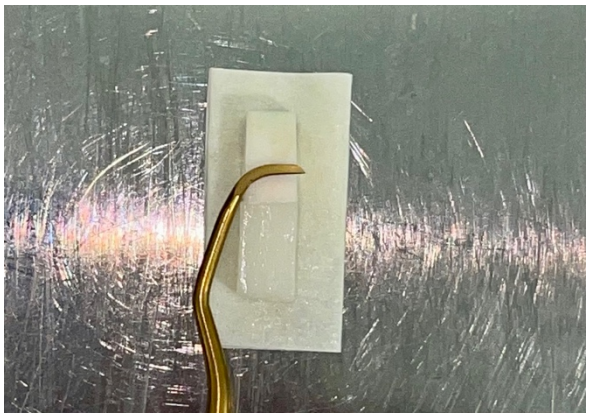


Figure 3.64 TiN curette instrumentation

[29] **The Double Gracey Anterior,** American Eagle Instruments ® Inc, Missoula, MT, USA

3.8. Ultrasonic Scaler Application

US application was performed on 20 specimens by a piezoelectric scaler[30] with the P1 tip[31] mounted (Figure 3.65-3.67)

The tip was kept parallel to the block surface and only the side surface of the tip was used (tip angulation was approximately 0-10°). External support was not used for angulation standardization since the instrument was at the minimum achievable angulation. A sweeping motion was adopted during instrumentation, the tip was kept constantly moving both in the longitudinal and transverse directions as the surface was traced starting from the base of the DS towards the CS. The application was performed for a duration of 60 seconds.

The handpiece was used at %100 of the operating performance (4 LED lights on) (The active electrical power of the instrument is 9.6 W). A constant stream of distilled water was delivered from the fluid container for cooling. The setting providing the highest consumption of water was selected (3 LEDs). 45ml of water was consumed per minute.



Figure 3.65.
Settings of ultrasonic scaler



Figure 3.66.
Ultrasonic scaler tip

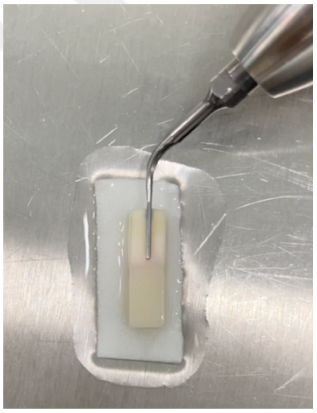


Figure 3.67.
Block instrumentation with ultrasonic scaler

[30] **Vector®Scaler**, Dürr Dental SE, Bietigheim-Bissingen, Germany
[31] **P1 tip,** Dürr Dental SE, Bietigheim-Bissingen, Germany

3.9. Linear Oscillating Device Application

The LOD[32] was applied on 20 specimens with the straight tip[33] together with a hydroxyapatite-based polishing fluid[34] during application as required for the device (Figure 3.68-3.72).

A sweeping motion was adopted during instrumentation, the tip was kept constantly moving both in the longitudinal and transverse directions as the surface was traced starting from the base of the DS towards the CS. The application was performed for a duration of 60 seconds.

The handpiece was used at %100 of the operating performance (5 LED lights on) (The active electrical power of the instrument is 9.6 Watts (W)). A pulsating jet of hydroxyapatite-based polishing fluid was delivered from the fluid bag in addition to distilled water from the water container of the unit.



Figure 3.68.
Straight tip of the linear oscillating device mounted on the resonating ring



Figure 3.69.
Linear oscillating device

[32] **Vector®Paro Pro,** Dürr Dental SE, Bietigheim-Bissingen, Germany
[33] **Paro probe straight tip,** Dürr Dental SE, Bietigheim-Bissingen, Germany
[34] **Vector® Fluid Polish,** Dürr Dental SE, Bietigheim-Bissingen, Germany



Figure 3.70. Polishing fluid



Figure 3.71. Instrumentation with linear oscillating device

3.10. Air Polisher Application - *(supra nozzle / grain size: ≈65 µm)*

An AP device[35] was used with a coarse trehalose APP[36] (≈65µm grain size) on 20 specimens. Supra nozzle and blue colored powder container was mounted on the jet unit. The handpiece was connected to the dental unit (Figure 3.72-3.74).

A personalized arm stand was made for the operator for standardization of AP application (Figure 3.75). An impression of the right forearm of the operator holding the AP in the desired position was taken starting from the wrist and ending at the elbow. The impression was mounted on a stiff box. Two grooves were carved on the arm stand. From these grooves, the arm stand was stabilized to the operation tray of the dental unit using two metal c-clamps.

The block was fixed on a metal tray with a piece of double-sided tape[37]. The operator used the personalized arm stand during instrumentation. The distance was set to 4mm between the tip and the block. The angulation during application was adjusted to ≈60° with an adjunctive device customized to guide the nozzle of the AP (Figure 3.76). Sweeping motions were made for 20 seconds (44). To increase the reproductivity of the power emission

[35] **MyLUNOS®,** Dürr Dental SE, Bietigheim-Bissingen, Germany
[36] **Lunos® Prophy Powder Gentle Clean,** Dürr Dental SE, Bietigheim-Bissingen, Germany
[37] **3M™ Double Coated Urethane Foam Tape 4016,** 3M, St. Paul., Minnesota, USA

of the instrument, the powder container was filled to the maximum powder level after each application (153).

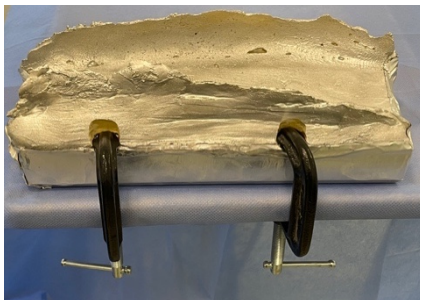


Figure 3.72.
Personalized arm stand



Figure 3.73.
Air polisher with the supra nozzle mounted



Figure 3.74.
Supragingival polishing powder



Figure 3.75.
Position of the arm of the operator during instrumentation

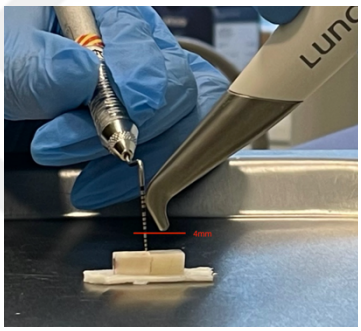


Figure 3.76.
Position of the tip of the air

3.11. Air Polisher Application *(perio nozzle with perio tip / grain size: ≈30 µm)*

An AP device[38] was used with a fine trehalose APP[39] (≈30µm grain size) on 20 specimens. Perio nozzle and orange colored perio-powder container was mounted to the jet unit (Figure 3.77-3.78). A new single-use perio tip was attached before starting the procedure

[38] **MyLUNOS® Perio**, Dürr Dental SE, Bietigheim-Bissingen, Germany
[39] **Lunos® Prophy Powder Perio Combi®**, Dürr Dental SE, Bietigheim-Bissingen, Germany

on each new block and the old one was discarded. The handpiece was connected to the dental unit. The block was fixed on a metal tray with a piece of double-sided tape.

During instrumentation the perio tip was kept parallel to the surface at all times and was in contact, however, no lateral pressure was applied (Figure 3.79). Sweeping motions were used as the tip was moved for 20 seconds (44) starting from the DS towards the CS. To increase the reproductivity of the power emission of the instrument, the powder container was filled to the maximum powder level after each application (153).



Figure 3.77.
Air polisher with the perio nozzle mounted



Figure 3.78.
Subgingival polishing powder

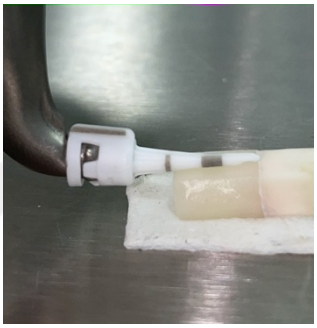


Figure 3.79.
Air polisher application

3.12. Surface Roughness Analysis

The sequences of SR analysis were like the following (Figure 3.80):
-Prior to cementation (DS, CS)
-After cementation (DS, resin cement surface, CS)
-After polishing (Baseline) (DS, resin cement surface, CS)
-After periodontal instrumentation (DS, resin cement surface, CS)

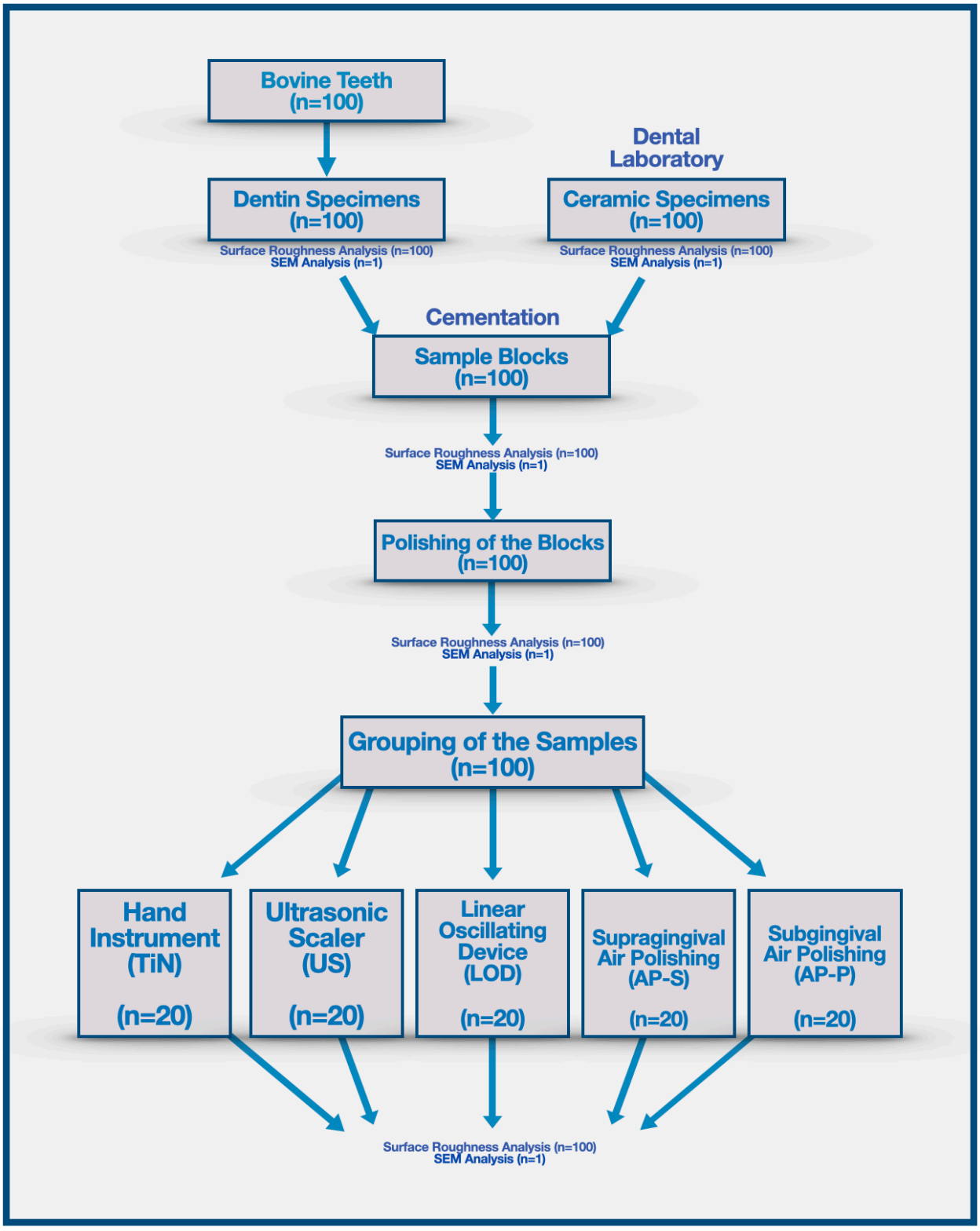


Figure 3.80. Sequences of surface roughness and SEM analyses

Before measurements were taken, the profilometer[40] was calibrated by using a metal block surface roughness tester (Mahr GmbH, Göttingen, Germany) (Figure 3.81). Profilometric measurements were taken by a blind examiner (OLT). Five individual measurements were recorded in different directions and from different locations (randomly and without tracing the same location twice) (84,211,212) on the specimens and a mean value of Ra for each surface (Ra; μm) was calculated (Cutoff 0.25mm, traversing length 0.8mm and tracing speed 0.1 mm/sec) by averaging the 5 values obtained (Figure 3.82-3.83).

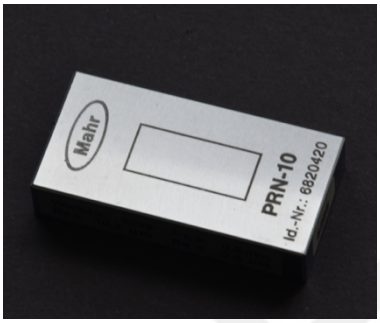


Figure 3.81
Metal surface roughness tester block



Figure 3.82.
Roughness measurement from the ceramic surface

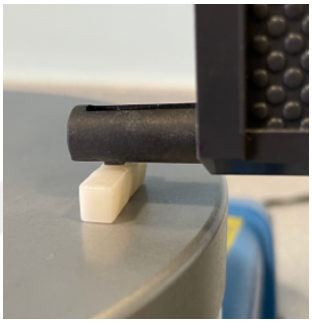


Figure 3.83.
Roughness measurement from the resin cement surface

3.13. Scanning Electron Microscope Analysis

The sequences of SEM analysis were like the following (Figure 3.80):
-Prior to cementation (DS, CS)
-After cementation ((DS, resin cement surface, CS)
-After polishing (Baseline) (DS, resin cement surface, CS)
-After periodontal instrumentation (DS, resin cement surface, CS)

An extra block for SEM representation was prepared with the same protocol for each step and each instrumentation method. SEM blocks were fixated by keeping them in 0.1M phosphate buffered saline solution containing %2.5 glutaraldehyde (pH 7.2) at room

[40] **Perthometer M1** Mahr GmbH, Göttingen, Germany

temperature for 24 hours and then washed with distilled water. All specimens were dried with air spray.

For platinum coating, SEM blocks were fixed on aluminum modules with double-sided carbon adhesive tape[41] and coded. The specimens were placed inside a sputter coater (Polaron Sputter Coater SC7659, Quorum Technologies, Newhaven, United Kingdom) and were coated with a fine layer (20 nm) of platinum (time: 90 seconds, current:10mA, vacuum:10^{-3} Torr). Platinum covered specimens were then placed in the vacuum chamber of the SEM. The SEM was operated at 10kV and 0° tilt angle. For the standardization of SEM microphotograph locations, the intersection of two diagonal lines crossing at the mid-points of the (i) entire block surface for resin cement junction, and separately for (ii) the DS and (iii) CS surfaces were located to be examined under x20 magnification. These 3 intersection points were further zoomed at to obtain microphotographs under higher magnifications of X75, X500, X750, x1000 and x2000 for inspecting the changes in surface morphology characteristics. The evaluation of the representative SEM images were conducted by a blind examiner (OLT).

3.14. Statistical Analysis

Data was analyzed on SPSS 25.0 (Statistical Packages of Social Sciences) program. For continuous variables, descriptive statistics were shown as arithmetic mean ± SD, median, minimum and maximum values. Normalite distribution assumption was evaluated with Shapiro-Wilks test. If surface changes of the specimens suited normal distribution and homogeneity of variance ANOVA test was used, and for variables which were found to be statistically significant, pairwise comparisons were done by Dunnett's T3 test. If it violated the assumption of homogeneity of variance then Kruskal Wallis test was used. For the evaluation of normally distributed measurements of changes pairwise over time, paired sample t-test was used, İf it violated the normally distributed then Wilcoxon Signed Rank test was used. Statistical significance was assessed at $p<0.05$.

[41] **Adhesive Carbon Tape,** Shinto Paint Company, Limited, Hyōgo, Japa

4.RESULTS

4.1. Surface Roughness Analysis Findings

4.1.1. Baseline (Polished After Cementation) Data of the Groups

For tooth, ceramic and resin cement surfaces, the inter-group comparisons of the mean Ra values at the baseline were conducted and the following results were reached.

4.1.1.1. Tooth Surfaces

Table 4.1. Inter-group comparison of mean Ra values of tooth surfaces at the baseline

	Baseline Ra (µm)	P value
TiN	0,142 ±0,040	[a]p =0,747
US	0,128 ±0,021	
LOD	0,137 ±0,033	
AP-S	0,129 ±0,032	
AP-P	0,138 ±0,027	

[a]Kruskal Wallis test, p<0.05

For tooth surfaces, the inter-group comparison of mean Ra values at the baseline did not reveal a significant difference between the groups (p=0,747) (Table 4.1).

4.1.1.2. Ceramic Surfaces

Table 4.2. Inter-group comparison of mean Ra values of ceramic surfaces at the baseline

	Baseline Ra (µm)	P value
TiN	0,221 ±0,049	[a]p =0,072
US	0,208 ±0,038	
LOD	0,253 ±0,071	
AP-S	0,202 ±0,053	
AP-P	0,213 ±0,050	

[a]Kruskal Wallis test, p<0.05

For ceramic surfaces, the inter-group comparison of mean Ra values at the baseline did not reveal a significant difference between the groups (p=0,072) (Table 4.2).

4.1.1.3. Resin Cement Surfaces

Table 4.3. Inter-group comparison of mean Ra values of resin cement surfaces at the baseline

	Baseline Ra (µm)	P value
TiN	0,942 ±0,051	[a]p =0,306
US	0,942 ±0,057	
LOD	0,965 ±0,068	
AP-S	0,922 ±0,086	
AP-P	0,933 ±0,049	

[a]ANOVA test, p<0.05

For resin cement surfaces, the inter-group comparisons of mean Ra values at the baseline did not reveal a significant difference between the groups (p=0,306) (Table 4.3).

4.1.2. Comparison of Surface Roughness After Periodontal Instrumentation

4.1.2.1. Instrumentation with TiN curette

When blocks were instrumented with the TiN curette after the baseline the following results were reached regarding the Ra values of intra-group changes (Table 4.4).

Table 4.4. Ra measurements before and after instrumentation with TiN curette

	Before Ra (µm)	After Ra (µm)	P value
Tooth Surface	0,142 ±0,040	0,202 ±0,041	p =0,001*
Ceramic Surface	0,221 ±0,049	0,235 ±0,046	p=0,066
Resin Cement Surface	0,942 ±0,051	1,082 ±0,078	p=0,000*

Paired Samples t Test, p<0.05

For tooth surfaces, Ra means at the baseline and after instrumentation with TiN curette were 0,142±0,040µm and 0,202±0,041µm respectively. Instrumentation of tooth surfaces with TiN curette increased the Ra value significantly (p=0,001, p<0,05).

For ceramic surfaces, Ra means at the baseline and after instrumentation with TiN curette were 0,221±0,049µm and 0,235±0,046µm, respectively. Instrumentation of ceramic surfaces with TiN curette did not increase the Ra value significantly (p=0,066, p<0,05).

For resin cement surfaces, Ra means at the baseline and after instrumentation with TiN curette were 0,942±0,051µm and 1,082±0,078µm respectively. Instrumentation of resin cement surfaces with TiN curette increased the mean Ra significantly (p=0,000, p<0,05).

4.1.2.2. US Application

When US application was conducted on the blocks after the baseline, the following results were reached regarding the intra-group Ra changes (Table 4.5).

Table 4.5. Ra measurements before and after instrumentation with US

	Before Ra (µm)	After Ra (µm)	P value
Tooth Surface	0,128 ±0,021	0,383 ±0,027	p =0,000*
Ceramic Surface	0,208 ±0,038	0,283 ±0,034	p=0,000*
Resin Cement Surface	0,942 ±0,057	1,156 ±0,064	p=0,000*

Paired Samples t Test p<0,05

For tooth surfaces, ceramic surfaces and resin cement surfaces, mean Ra values at the baseline increased significantly after the application of US (p=0,000, p=0,000, p=0,000, respectively) (p<0,05).

4.1.2.3. LOD Application

When LOD application was conducted on the blocks after the baseline, the following results were reached regarding the intra-group Ra changes (Table 4.6).

Table 4.6. Ra measurements before and after instrumentation with the LOD

	Before Ra (µm)	After Ra (µm)	P value
Tooth Surface	0,137 ±0,033	0,367 ±0,062	p =0,000*
Ceramic Surface	0,253 ±0,071	0,283 ±0,070	p=0,034*
Resin Cement Surface	0,965 ±0,068	1,108 ±0,063	p=0,000*

Paired Samples t Test p<0,05

For tooth surfaces, ceramic surfaces and resin cement surfaces, mean Ra values at the baseline increased significantly after the application of LOD (p=0,000, p=0,034, p=0,000, respectively) (p<0,05).

4.1.2.4. AP-S Application *(supra nozzle / grain size ≈65 µm)*

When AP-S application was conducted on the blocks after the baseline, the following results were reached regarding the intra-group Ra changes (Table 4.7).

Table 4.7. Ra measurements before and after AP-S application

	Before Ra (µm)	After Ra (µm)	P value
Tooth Surface	0,129 ±0,032	0,278 ±0,053	p =0,000*
Ceramic Surface	0,202 ±0,053	0,233 ±0,051	p=0,004*
Resin Cement Surface	0,922 ±0,086	1,262 ±0,062	p=0,000*

Paired Samples t Test p<0,05

For tooth surfaces, ceramic surfaces and resin cement surfaces, mean Ra values at the baseline increased significantly after the application of AP-S (p=0,000, p=0,004, p=0,000, respectively) (p<0,05).

4.1.2.5. AP-P Application *(perio nozzle / grain size ≈30 µm)*

When AP-P application was conducted on the blocks after the baseline, the following results were reached regarding the intra-group Ra changes (Table 4.8).

Table 4.8. Ra measurements before and after AP-P application

	Before Ra (µm)	After Ra (µm)	P value
Tooth Surface	0,138 ±0,027	0,204 ±0,032	p =0,000*
Ceramic Surface	0,213 ±0,050	0,225 ±0,050	p=0,481
Resin Cement Surface	0,933 ±0,049	1,167 ±0,119	p=0,001*

Paired Samples t Test p<0,05

For tooth surfaces, Ra at the baseline and after AP-P application were 0,138±0,027µm and 0,204±0,032µm, respectively. AP-P application on tooth surfaces increased the Ra value significantly (p =0,000, p<0,05).

For ceramic surfaces, Ra the baseline and after AP-P application were 0,213±0,050µm and 0,225±0,050µm, respectively. AP-P application on ceramic surfaces did not increase the Ra value significantly (p=0,481, p<0,05).

For resin cement surfaces, Ra at the baseline and after AP-P application were 0,933±0,049µm and 1,167±0,119µm, respectively. AP-P application on resin cement surfaces increased the Ra value significantly (p=0,001, p<0,05).

4.1.3. Inter-group Comparison of the Mean Intra-group Changes in Surface Roughness After Periodontal Instrumentation

4.1.3.1. Tooth Surfaces

When the intra-group mean Ra changes of the tooth surfaces were compared between the groups, a statistically significant difference was found (p=0,000, p<0.05) (Table 4.9).

Table 4.9. Multiple comparison of the intra-group Ra changes of tooth surfaces after periodontal instrumentation

Group Differences	n	Mean ±SD (µm)	Median (µm)	Minimum (µm)	Maximum (µm)	P value
TiN	20	0,060 ±0,033	0,064	0,003	0,118	0,000*
US	20	0,254 ±0,035	0,253	0,181	0,321	
LOD	20	0,230 ±0,059	0,229	0,158	0,358	
AP-S	20	0,149 ±0,060	0,147	0,038	0,281	
AP-P	20	0,065 ±0,039	0,055	-0,001	0,135	

Welch ANOVA test, p<0,05

Table 4.10. Pairwise comparisons of the mean average Ra changes of tooth surfaces after periodontal instrumentation

Pairwise Comparisons of Ra Changes of Tooth Surfaces After Periodontal Instrumentation					
(A-B)	**TiN** (B) **(µm)**	**US** (B) **(µm)**	**LOD** (B) **(µm)**	**AP-S** (B) **(µm)**	**AP-P** (B) **(µm)**
TiN (A) **(µm)**		-0,195* ±0,011	-0,170* ±0,015	-0,089* ±0,015	-0,006 ±0,011
US (A) **(µm)**	0,195* ±0,011		0,024 ±0,015	0,105* ±0,016	0,189* ±0,012
LOD (A) **(µm)**	0,170* ±0,015	-0,024 ±0,015		0,081* ±0,019	0,165* ±0,016
AP-S (A) **(µm)**	0,089* ±0,015	-0,105* ±0,016	-0,081* ±0,019		0,084* ±0,016
AP-P (A) **(µm)**	0,006 ±0,011	-0,189* ±0,012	-0,165* ±0,016	-0,084* ±0,016	

Dunnet T3 test, p<0,05

Negative (-) values indicate that the Ra value change caused by the B group is greater than the Ra value caused by the A group

The multiple comparison of the intra-group changes revealed a statistical significance (Table 4.9). Pairwise comparison conducted for this statistical difference showed that the instrumentation method causing the highest increase in the mean Ra value was the US group, second highest was the LOD group followed by the AP-S, AP-P and TiN groups (TiN < AP-P < AP-S < LOD < US). TiN group revealed the least change in SR. Along with the pairwise inter-group comparisons, statistical significant differences were found between the groups: (TIN-US)(p=0,000 p<0,05)(95% confidence interval (CI) [-0,226; -0,163]), (TiN-LOD)(p=0,000 p<0,05)(95% CI [-0,215; -0,125]), (TiN-AP-S)(p=0,000, p<0,05)(95% CI [-0,135; -0,043]), (US-AP-S)(p=0,000 p<0,05)(95% CI [0,059; 0,152]), (US-AP-P)(p=0,000 p<0,05)(95% CI [0,154; 0,224]), (LOD-AP-S)(p=0,001 p<0,05)(95% CI [0,026; 0,137]), (LOD-AP-P)(p=0,000 p<0,05)(95% CI [0,118; 0,212]), (AP-S-AP-P)(p=0,000 p<0,05)(95% CI [0,036; 0,131]) except for the (TiN-AP-P)(p=1,000 p<0,05)(95% CI [0,040; 0,283]) and (US-LOD)(p=0,688 p<0,05)(95% CI [-0,021; 0,070]) (Table 4.10).

4.1.3.2. Ceramic Surfaces

When the intra-group mean Ra changes of the ceramic surfaces were compared between the groups, a statistically significant difference was found (p=0,000 p<0.05) (Table 4.11).

Table 4.11. Multiple comparison of the intra-group Ra changes of ceramic surfaces after periodontal instrumentation

Group Differences	n	Mean ±SD (µm)	Median (µm)	Minimum (µm)	Maximum (µm)	P value
TiN	20	0,014 ±0,031	0,014	-0,040	0,070	0,000*
US	20	0,075 ±0,039	0,074	0,010	0,130	
LOD	20	0,030 ±0,058	0,025	-0,070	0,140	
AP-S	20	0,030 ±0,042	0,027	-0,060	0,120	
AP-P	20	0,012 ±0,076	0,028	-0,160	0,130	

Welch ANOVA test was conducted. *p<0,05 statistically significant

Table 4.12. Pairwise comparisons of the mean average Ra changes of ceramic surfaces after periodontal instrumentation

Pairwise Comparisons of the Mean Average Ra Changes of Ceramic Surfaces After Periodontal Instrumentation					
(A-B)	**TiN** (B) (µm)	**US** (B) (µm)	**LOD** (B) (µm)	**AP-S** (B) (µm)	**AP-P** (B) (µm)
TiN (A) (µm)		-0,061* ±0,011	-0,016 ±0,015	-0,017 ±0,012	0,001 ±0,018
US (A) (µm)	0,061* ±0,011		0,045 ±0,016	0,045* ±0,013	0,063* ±0,019
LOD (A) (µm)	0,016 ±0,015	-0,045 ±0,016		-0,001 ±0,016	0,017 ±0,021
AP-S (A) (µm)	0,017 ±0,012	-0,045* ±0,013	0,001 ±0,016		0,018 ±0,019
AP-P (A) (µm)	-0,001 ±0,018	-0,063* ±0,019	-0,017 ±0,021	-0,018 ±0,019	

Dunnet T3 test, p<0,05

Negative (-) values indicate that the Ra value caused by the B group is greater than the Ra value caused by the A group

The multiple comparison of the intra-group changes revealed a statistical significance (Table 4.11). The instrumentation method causing the highest increase in the mean Ra value was the US group, second highest was the AP-S group followed by the LOD, TiN and AP-P groups (AP-P < TiN < LOD < AP-S < US). AP-P group revealed the least change in SR. When the pairwise intra-group comparisons were conducted, statistical significant differences were found between the groups: (TIN-US)(p=0,000 p<0,05)(95% CI [-0,094; -0,029]), (US-AP-S)(p=0,012 p<0,05)(95% CI [0,007; 0,082]), (US-AP-P)(p=0,026 p<0,05)(95% CI [0,005; 0,120]) except for the (TIN-LOD)(p=0,951 p<0,05)(95% CI [-0,060; 0,028]), (TIN-AP-S)(p=0,793 p<0,05)(95% CI [-0,051; 0,018]), (TIN-AP-P)(p=1,000 p<0,05)(95% CI [-0,055; 0,057]), (US-LOD)(p=0,061 p<0,05)(95% CI [-0,001; 0,092]), (LOD-AP-S)(p=1,000 p<0,05)(95% CI [-0,048; 0,047]), (LOD-AP-P)(p=0,994 p<0,05)(95% CI [-0,046; 0,081]), (AP-S-AP-P)(p=0,983 p<0,05)(95% CI [-0,040; 0,077]) (Table 4.12).

4.1.3.3. Resin Cement Surfaces

When the intra-group mean Ra changes of the ceramic surfaces were compared between the groups, a statistically significant difference was found (p=0,000 p<0.05) (Table 4.13).

Table 4.13. Multiple comparison of the intragroup Ra changes of resin cement surfaces after periodontal instrumentation

Group Differences	n	Mean ±SD (µm)	Median (µm)	Minimum (µm)	Maximum (µm)	P value
TiN	20	0,140 ±0,094	0,135	-0,010	0,300	0,000*
US	20	0,213 ±0,086	0,203	0,080	0,380	
LOD	20	0,143 ±0,083	0,137	-0,040	0,300	
AP-S	20	0,340 ±0,107	0,351	0,180	0,550	
AP-P	20	0,233 ±0,132	0,226	0,000	0,620	

Welch ANOVA test was conducted, p<0,05

Table 4.14. Pairwise comparisons of the mean average Ra changes of resin cement surfaces after periodontal instrumentation

Pairwise Comparisons of the Mean Average Ra Changes of Resin Cement Surfaces After Periodontal Instrumentation					
(A-B)	**TiN** (B) **(µm)**	**US** (B) **(µm)**	**LOD** (B) **(µm)**	**AP-S** (B) **(µm)**	**AP-P** (B) **(µm)**
TiN (A) **(µm)**		-0,073 ±0,028	-0,003 ±0,028	-0,200* ±0,032	-0,093 ±0,036
US (A) **(µm)**	-0,073 ±0,028		-0,070 ±0,027	-0,127* ±0,031	-0,020 ±0,035
LOD (A) **(µm)**	0,003 ±0,028	-0,070 ±0,027		-0,197* ±0,030	-0,090 ±0,035
AP-S (A) **(µm)**	0,200* ±0,032	0,127* ±0,031	0,197* ±0,030		0,107 ±0,038
AP-P (A) **(µm)**	0,093 ±0,036	0,020 ±0,035	0,090 ±0,035	-0,107 ±0,038	

Dunnet T3 test was conducted, p<0,05

Negative (-) values indicate that the Ra value caused by the B group is greater than the Ra value caused by the A group

The multiple comparison of the intra-group changes revealed a statistical significance (Table 4.13). The instrumentation method causing the highest increase in the mean Ra value was the AP-S group, second highest was the AP-P group followed by the US, LOD and TiN groups (TiN < LOD < US < AP-P < AP-S). TiN group revealed the least change in SR. When the pairwise intra-group comparisons were conducted, statistical significant differences were found between the groups: (TiN-AP-S)(p=0,000 p<0,05)(95% CI [-0,295; -0,106]), (US-AP-S)(p=0,002 p<0,05)(95% CI [-0,218; -0,036]), (LOD-AP-S)(p=0,000 p<0,05)(95% CI [-0,287; -0,107]) except for the (TiN-US)(p=0,125 p<0,05)(95% CI [-0,158; 0,011]), (TiN-LOD)(p=1,000 p<0,05)(95% CI [-0,087; 0,080]), (TiN-AP-P)(p=0,131 p<0,05)(95% CI [-0,201; 0,015]), (US-LOD)(p=0,114 p<0,05)(95% CI [-0,001; 0,150]), (US-AP-P)(p=1,000 p<0,05)(95% CI [-0,125; 0,086]), (LOD-AP-P)(p=0,133 p<0,05)(95% CI [-0,195; 0,015]) and (AP-S-AP-P)(p=0,074 p<0,05)(95% CI [-0,006; 0,220]) (Table 4.14).

4.1.4. The Effect of Cementation on Surface Roughness Values

Previous to the planned experimental period, the Ra values during the preparation of the blocks to baseline, measurements before and after cementation were additionally compared for tooth and ceramic surfaces and a statistically significant difference was found (p=0,000, p=0,000, respectively) (p<0,05) (Table 4.15). Cementation increased the Ra values significantly.

Table 4.15. Ra changes before and after cementation

	Mean±SD (µm)	N	Std. Error Mean	P value
Tooth Surfaces (Before)	0,188±0,037	100	0,004	0,000*
Tooth Surfaces (After)	0,311±0,052	100	0,005	
Ceramic Surfaces (Before)	0,260±0,063	100	0,006	0,000*
Ceramic Surfaces (After)	0,317±0,065	100	0,006	

Paired samples t test p<0,05

4.1.5. The Effect of Polishing After Cementation on Surface Roughness Values

According to the experimental protocol, the blocks polished after cementation were the ones ready for the baseline characteristics. For additional interest, when the mean Ra values obtained after cementation were compared to after polishing (experimental baseline) for tooth, ceramic and resin cement surfaces, a statistically significant difference was found (^{a}p=0,000, ^{b}p=0,000, p^{a}=0,000, respectively) (p<0,05) (Table 4.16). Polishing after cementation dramatically smoothed the roughened surfaces due to cementation.

Table 4.16. Ra changes caused by polishing following cementation

	Mean±SD (µm)	N	Std. Error Mean	P value
Tooth Surface Before Polishing	0,311±0,052	100	0,005	**[a]p=0,000***
Tooth Surface After Polishing	0,135±0,031	100	0,003	
Ceramic Surface Before Polishing	0,317±0,065	100	0,006	**[b]p=0,000***
Ceramic Surface After Polishing	0,219±0,055	100	0,006	
Resin Cement Before Polishing	1,562±0,158	100	0,016	**[a]p=0,000***
Resin Cement After Polishing	0,941±0,064	100	0,006	

[a]Paired Samples t Test p<0,05

[b]Wilcoxon's Signed Rank Test p<0,05

4.2. SEM Findings

4.2.1. Representative SEM Images of Surfaces Before Cementation

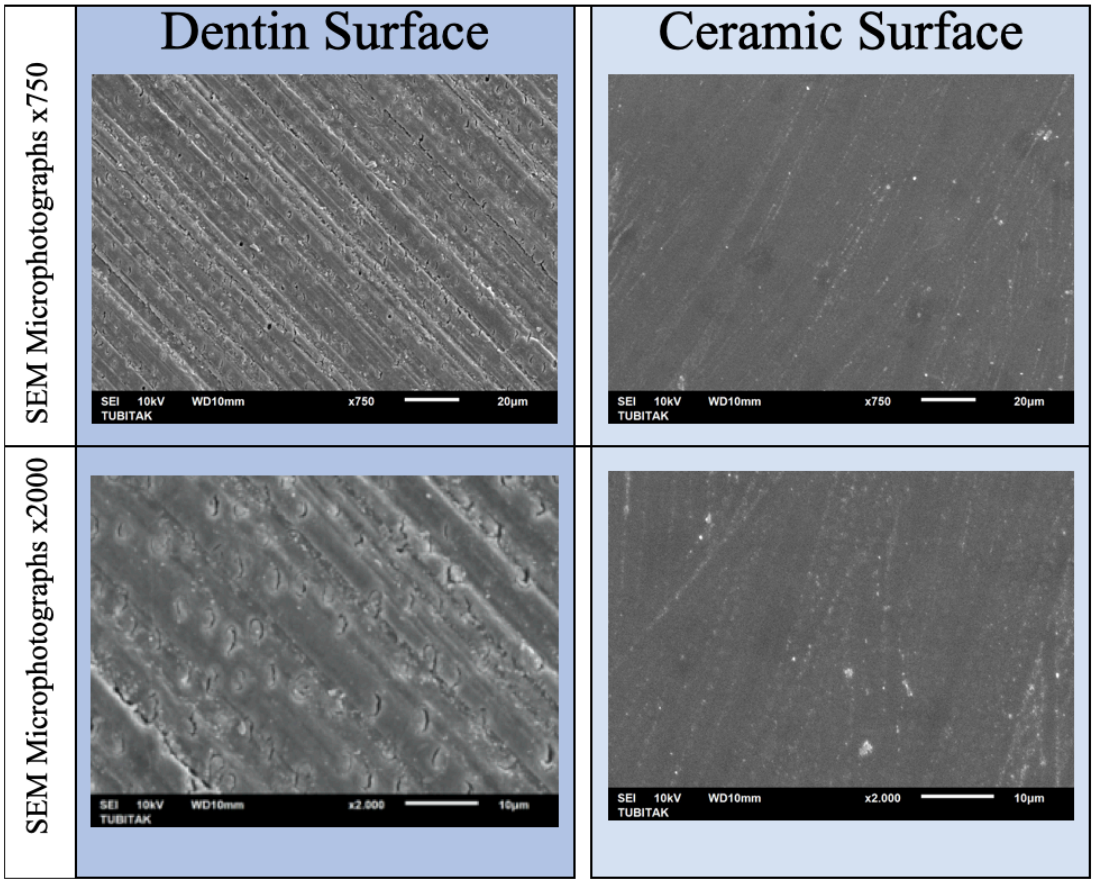


Figure 4.1. Dentin and ceramic surfaces prior to cementation (x750, x2000)

When the x750 microphotograph of the surface as immediately after tooth specimen preparation, thus SEM representative for dentin "before cementation" (Table 17) was evaluated, the linear marks due to wet polishing through silicon carbide paper were detected as well as the presence of dentin tubules. In the x2000 microphotograph occluded dentin tubule entrances were visible (Figure 4.1).

Before cementation, when the x750 microphotograph of unpolished ceramic surface was evaluated, a homogenous glazed surface was obvious (See Table 4.15 for numerical

values). In the x2000 microphotograph, since glaze application was done with a brush, marks from the brush strokes were visible (Figure 4.1).

4.2.2. Representative SEM Images of Surfaces Immediately After Cementation

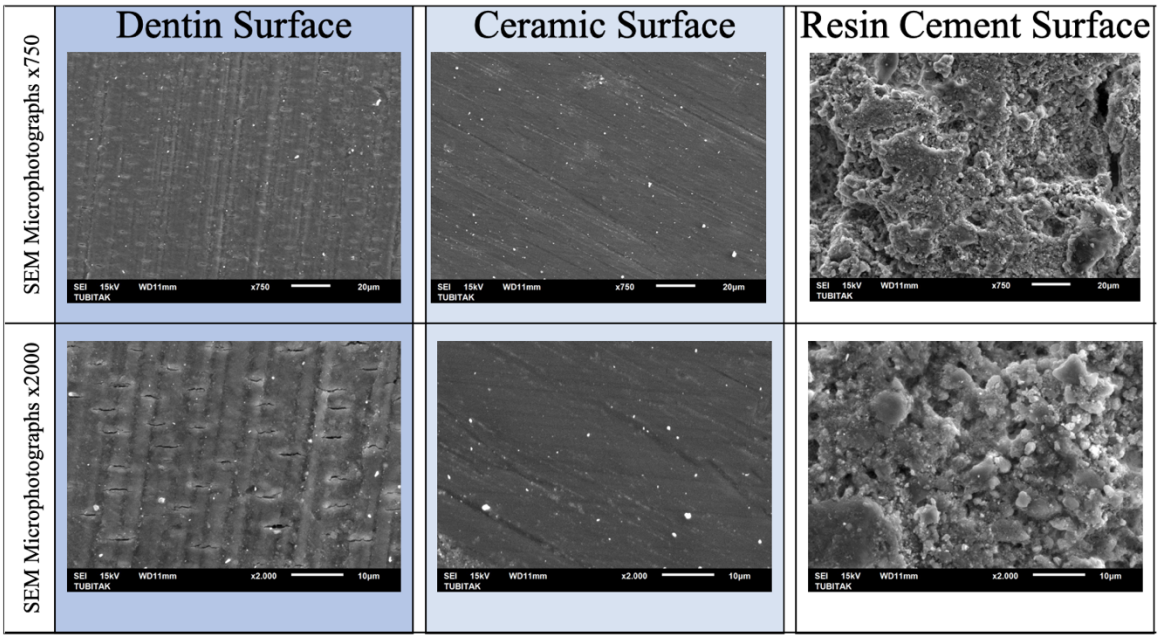


Figure 4.2. Dentin, ceramic and resin cement surfaces immediately after cementation (x750, x2000)

For representative dentin and ceramic surfaces, compared with x750 microphotographs previously obtained before cementation (Table 4.15), a notable difference was not observed for microphotographs obtained after cementation. However, when x2000 microphotographs for dentin and ceramic surfaces were evaluated, leftover residues from the cementation process concomitant with the increase in the profilometric analysis became noticeable (Table 4.16) (Figure 4.2).

When the representative resin cement surface was evaluated under both low (x750) and high (x2000) magnifications, a rough heterogenous surface topography was observed together with diffuse porosities throughout the surface (Table 4.16) (Figure 4.2).

4.2.3. Representative SEM Images of Surfaces After Polishing the Cemented Surfaces

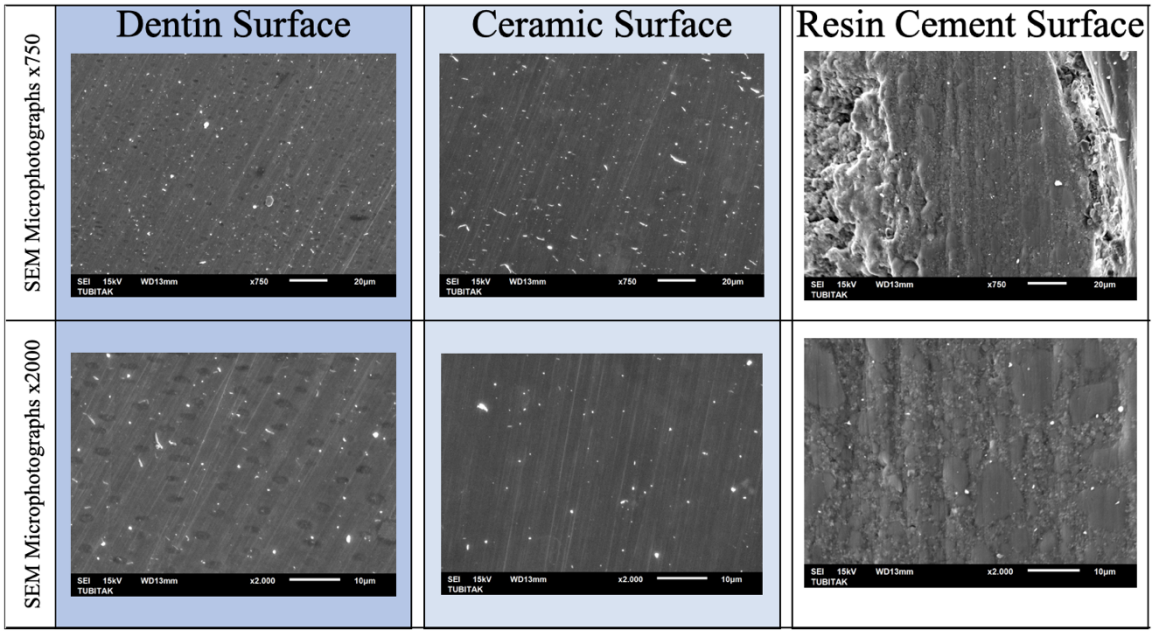


Figure 4.3. Dentin, ceramic and resin cement surfaces after polishing following cementation (x750, x2000)

On polished dentin surface throughout the representative sample, a smoother surface compared to the previous one (Table 4.16) and a fine smear layer was observed. Dentin tubules as blurred entrances were seen in x750 microphotograph. In x2000 magnification, residual particles from the polisher were detected. Dentin tubule entrances were covered with a fine tulle-like layer assumed to be the smear (Figure 4.3).

For representative ceramic surfaces under x750 magnification, a homogenous surface topography was observed after polishing (Table 4.16). In x2000 microphotographs, residual particles from the polisher were seen (Figure 4.3).

When the x750 microphotograph of the representative resin cement surface was evaluated, a homogenous surface topography was obtained in the polished areas (Table 4.16). However, in areas where the polisher was not able to access, diffuse porosities remained.

Under x2000 magnification, the particulated structure of the resin cement was observed as well as residual particles from the polisher (Figure 4.3).

4.2.4. Representative SEM Images of Dentin Surfaces After Periodontal Instrumentation

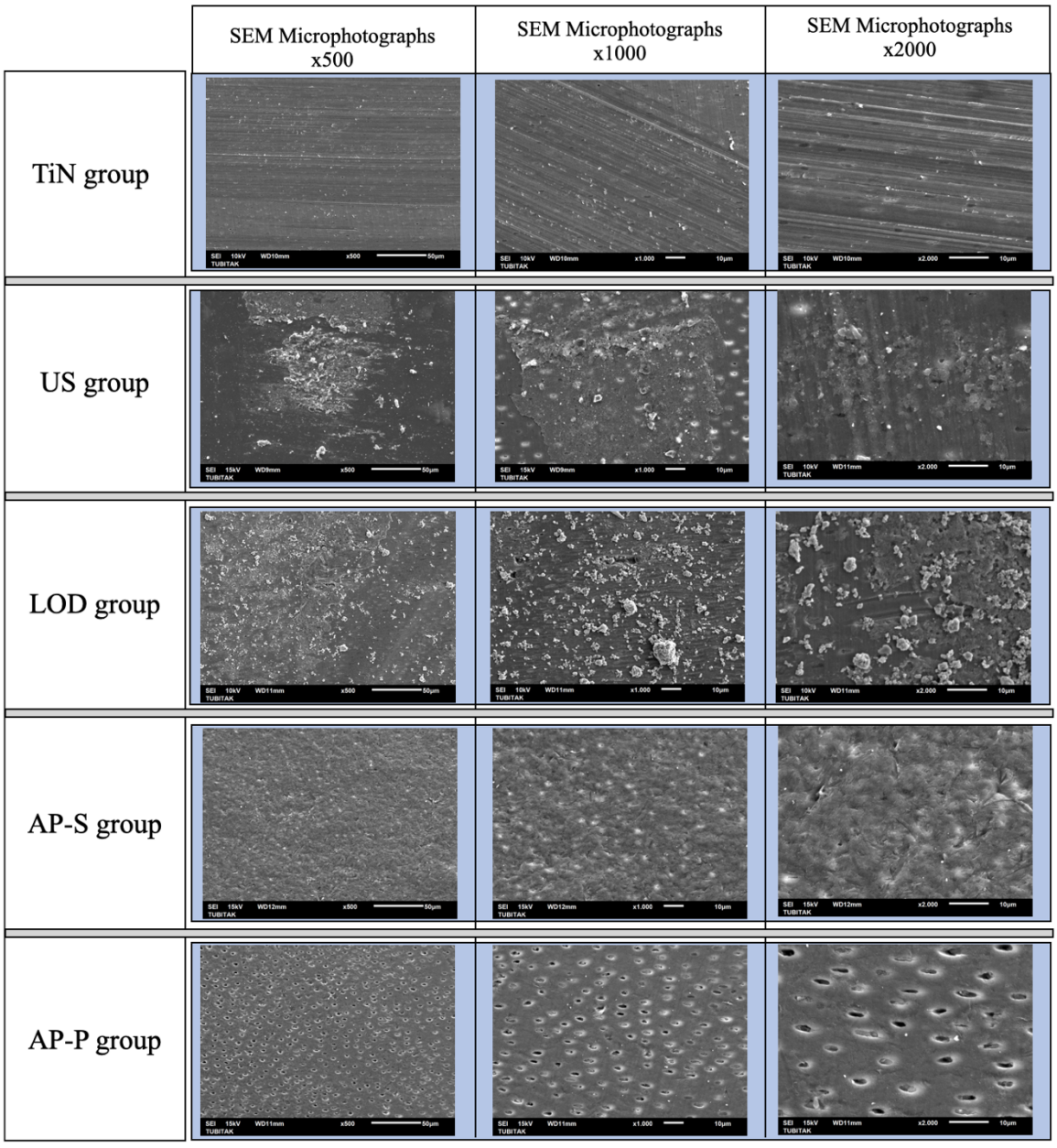


Figure 4.4. Dentin surfaces after periodontal instrumentation (x500, x1000, x2000)

On representative dentin surface instrumented with TiN curette, low magnification (x500) microphotographs revealed surfaces similar to the microphotographs obtained after cementation but before polishing the samples. However, at closer magnifications (x1000 and x2000), deeper scratch marks were seen at areas contacted with the curette. Also, it was seen that the dentin tubule entrances were less apparent in these samples (Figure 4.4).

The representative dentin surface instrumented with the US presented topographic irregularities on the surface caused by the working end of the instrument which were observed more clearly at x1000 and x2000 magnification. Also, on the surfaces where topographic changes are apparent, the presence of smear layer was seen. Presence of smear layer occluded the entrances of dentin tubules; therefore, no dentin tubule entrances were apparent (Figure 4.4).

On representative dentin surface instrumented with LOD, a dense and thick layer of hydroxyapatite particles was observed. The topography was homogenous. At closer magnifications (x1000 and x2000), the SR with indentations and protrusions became apparent. Also, at x2000 magnification, a defect resembling the shape of a long claw mark probably caused by the working end of the instrument was observed (Figure 4.4).

Application of AP-S to the representative dentin surface revealed a rough surface where indentations and protrusions were present at x500 and x1000 magnifications. However, even though the surface was rough, the surface texture seemed organized. When a closer magnification (x2000) was conducted, it was seen that the dentin tubules entrances were occluded (Figure 4.4).

When the microphotographs obtained after the application of AP-P were evaluated, the intertubular structure was found intact. AP-P application did not cause surface irregularities on the representative surfaces. It was also concluded that there was no smear formation in these microphotographs and the tubule entrances were found completely open (Figure 4.4).

4.2.5. Representative SEM Images of Ceramic Surfaces After Periodontal Instrumentation

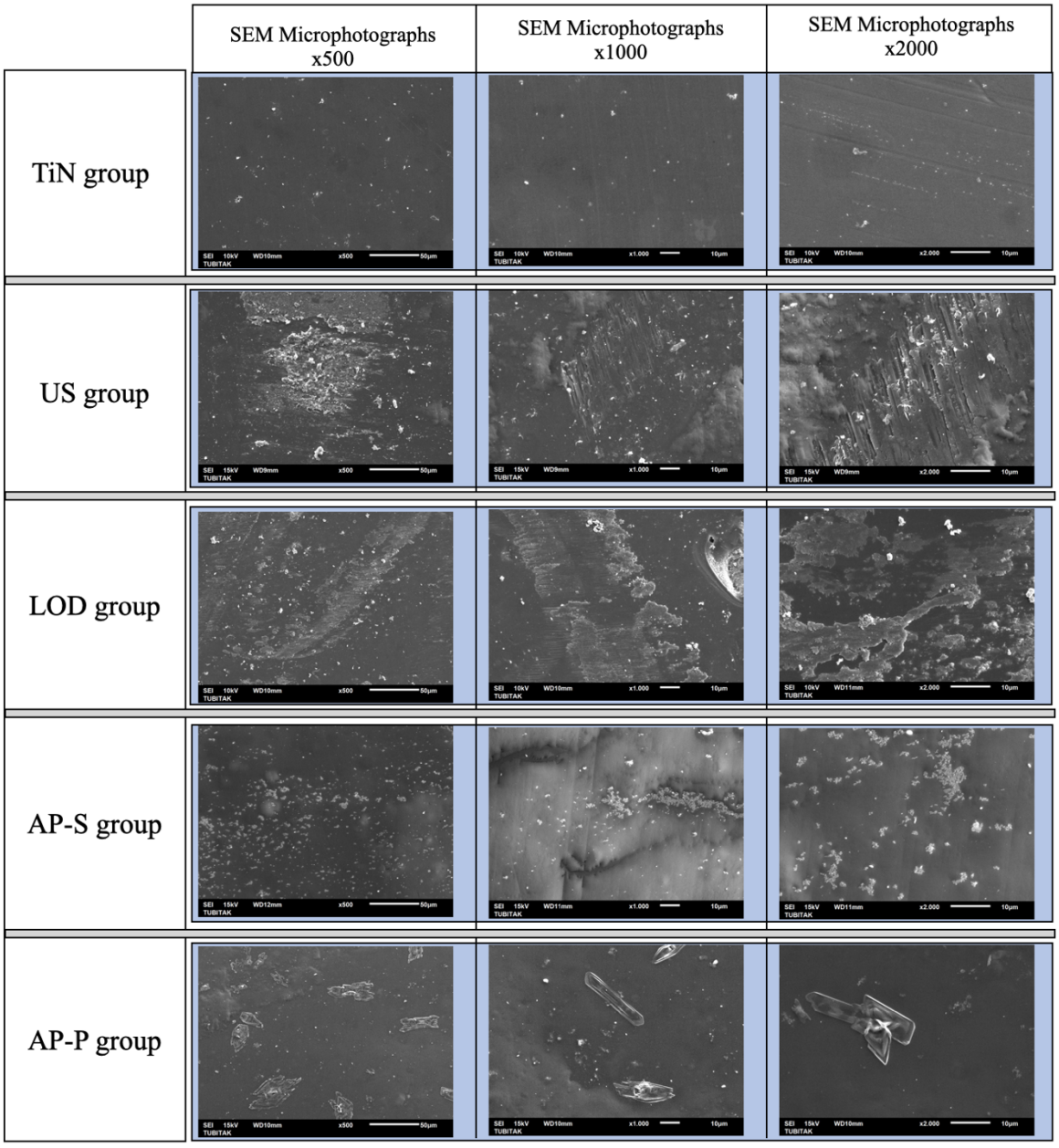


Figure 4.5. Ceramic surfaces after periodontal instrumentation (x500, x1000, x2000)

In the representative ceramic samples instrumented with the TiN curette, no observable differences from the baseline images were seen under low and high magnifications (x500, x1000 and x2000) (Figure 4.5).

When the representative ceramic surface was instrumented with the US, a disruption of the surface texture was seen as well as the presence of residual particles under x500 magnification. At a closer magnification scratch marks were clearly seen (x1000). At the highest magnification (x2000), scratch marks became in abundance. The depth of indentations and height of the protrusions were pronounced (Figure 4.5).

On the representative ceramic samples instrumented with the LOD, residues of hydroxyapatite particles were seen under low magnification (x500) as well as cavity like substance removal (x1000). Furthermore, a surface characteristic with a peeled appearance was prominent, possibly due to the straight probe tip of the LOD causing scratches on the surface. The homogeneity of the ceramic surface was disrupted (both on the x1000 and x2000 magnifications) (Figure 4.5).

When the microphotographs of representative ceramic surfaces were evaluated after application of AP-S, it was observed that there was a disruption in surface texture under low magnification (x500). When x1000 microphotograph was examined, it was detected that the homogeneity of the surface was altered, and the glaze was removed superficially. The microphotograph x2000 revealed stapled like multiple distortions of the surface (Figure 4.5).

Representative ceramic samples underwent AP-P application, showed no obvious differences from the baseline under low and high magnifications (x500, x1000 and x2000). Under all magnifications trehalose crystals were seen all over the surfaces (Figure 4.5).

4.2.6. Representative SEM Images of Resin Cement Surfaces After Periodontal Instrumentation

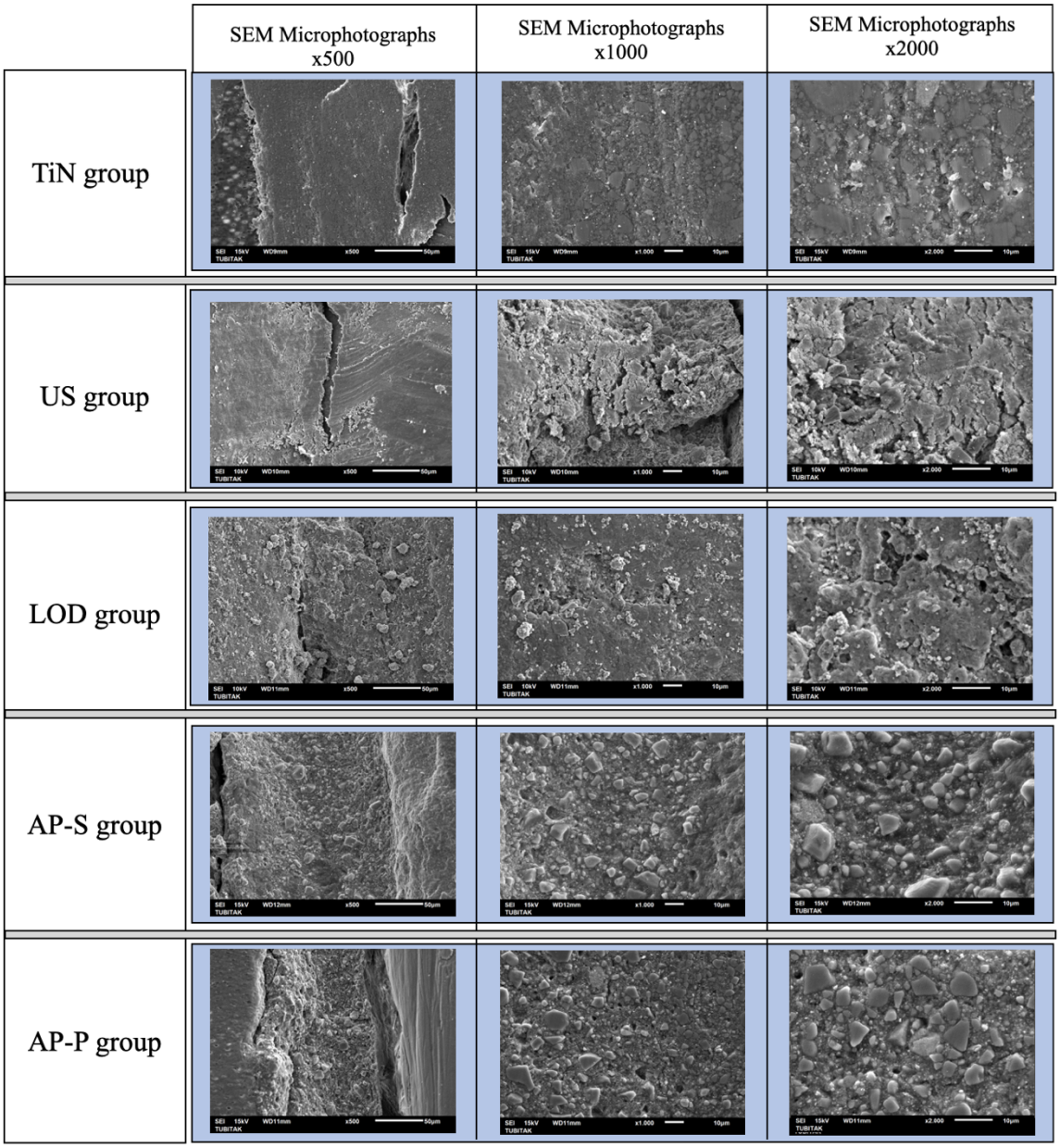


Figure 4.6. Resin cement surfaces after periodontal instrumentation (x500, x1000, x2000)

When the representative resin cement surface instrumented with the TiN curette was evaluated under x500 magnification, a smooth surface topography was observed. At closer magnifications (x1000 and x2000), an organized particulated structure of the cement became apparent (Figure 4.6).

On representative resin cement surfaces instrumented with the US, scratch marks were observed. At higher magnifications (x1000 and x2000) cracked form of structures were observed (Figure 4.6).

After instrumentation with LOD, when x500 microphotographs were viewed, the presence of hydroxyapatite particles was apparent. Also, beneath the particles, the topographic surface differences caused by the working end of the instrument were seen at x1000 magnification. At x2000, it was observed that the representative resin cement surface lying beneath the hydroxyapatite particles was heterogenous in texture and diffusely porous (Figure 4.6).

When APs were applied to the representative resin cement surface, for both AP-S and AP-P at x500 magnification, the resin was found to be partially removed revealing a clear outline of the glass filler particles of the resin material. At closer magnifications (x1000 and x2000), the glass particles became more obvious in the AP-S group versus the AP-P group (Figure 4.6).

4.2.7. Representative SEM Images of Interfaces After Instrumentation

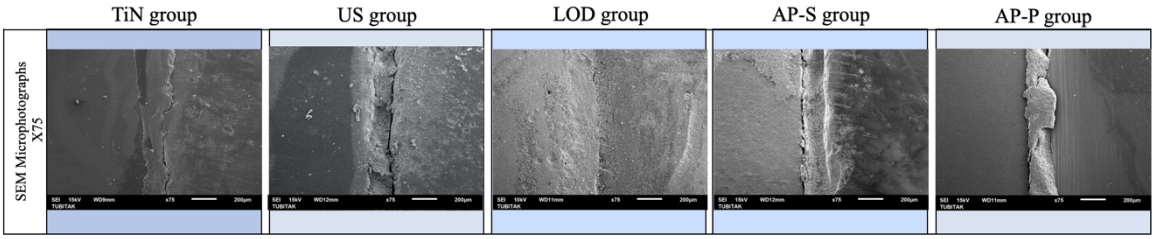


Figure 4.7. Tooth, resin cement, ceramic interfaces after periodontal instrumentations (x75)

In the previous sections, the effect of various periodontal instrumentation modalities and their effects on each one of the representative surfaces resembling the restoration-tooth interface was given in details. In these SEM microphotographs the tooth, resin cement, ceramic interface can be seen at a lower magnification (x75) (Figure 4.7). The left side of the microphotographs is the dentin surface, the right side is the ceramic surface and the surface

in between is the resin cement surface. For tooth and ceramic surfaces, instrumentation with TiN curette and application of AP-P revealed the most homogenous and smooth surfaces. However, for the resin cement surface, besides instrumentation with TiN curette, LOD application presented the most acceptable surface characteristics.

5. DISCUSSION

Margins of restorations are susceptible to plaque accumulation and periodontal disease (64,67-71). Placement of fixed prosthetic restorations have increased over the years (176) and also results in an increase in the number of margins in the oral cavity. For maintaining periodontal health after placement of fixed prosthetic restorations, periodontal interventions are essential (64-71,201,202).

The aim of this *in vitro* study was to evaluate the effects of different periodontal tools and mechanical instrumentation on the tooth restoration interface. The representative tooth restoration interfaces prepared on bovine central incisors were instrumented with a TiN curette, an US, the LOD, an AP with a supra nozzle using a ≈*65µm* APP and an AP with a perio nozzle using a ≈30 *µm* APP for surface evaluation.

Regardless of the instrumentation type; an increase of Ra was observed on all the surfaces constituting the interface. The only instrument-surface combinations where this increase was not significant were the ceramic surfaces instrumented with the TiN curette and AP with the perio nozzle using a ≈30*µm* APP. It was concluded that while instrumenting ceramic surfaces, TiN curette and AP device with a perio nozzle and a fine APP may be the instruments of choice.

The Ra of all the dentin surfaces were measured to be below the SR threshold value (Ra: 0.2µm) (51,57) at the baseline (after polishing) without significant difference ($p<0.05$). After all types of instrumentation, Ra representing SR, raised above the threshold value together with the possibility of leading to clinical consequences such as increases in bacterial colonization on the surfaces. For ceramic surfaces and resin cement surfaces; the initial mean Ra values were measured to be already above the SR threshold value and a further increase was seen after instrumentation.

In our study, DSs were obtained from the central teeth of approximately 3 years old freshly slaughtered cattle. The rationale behind the use of non-human tooth substances is the

requirement for flat surfaces of uniform thickness in certain tests where the small and curved surface areas of human teeth can be a limiting factor (213). Also, the requirement to find the needed number of adequately qualified teeth can become a challenge for the researchers. Most human teeth are extracted either due to caries lesions or other defects which may make them ineligible for being used in research (214). Bovine teeth in good condition can be obtained in large numbers and their composition is more uniform compared to human teeth. They have large flat surfaces with no caries lesions and/or defects which may have a jeopardizing effect on outcome measures (83).

In data collection, standardization carries an utmost importance. When human teeth are used, the source and age of the teeth can become very hard to control and lack of such control may lead to large variations (213). It has been reported that as the age of an individual increases, mineralization of the dentin increases (215). Also with aging, the dentinal tubules gradually thicken resulting in a reduction of the tubule diameters, making the dentin become harder, denser and less sensitive due to the restricted fluid flow in the tubules (74). Another concern regarding the use of human teeth is infection hazard (216) and increased ethical concerns (217).

Mostly lower anterior bovine teeth are used for research purposes due to their large size and flat surfaces. Anterior teeth are comprised of three pairs of incisors and a single pair of canines (218). In our study, the radicular dentin surfaces of central teeth were used which are the largest bovine teeth. This enabled a standardization of the periodontal instrumentation applied on the blocks and also a detailed evaluation of the surface characteristics. Since a contact profilometer was used, a large surface area was beneficial and appropriate.

Several studies made comparisons between permanent bovine and human teeth dentin; Imfield (219) concluded that the use of bovine mandibular front teeth was eligible for *in vitro* studies when the results regarding SR and abrasion were evaluated. Wegehaupt et al. (220) concluded that there was not a statistically different level of dentin wear between human and bovine samples and in abrasion tests cattle's or calf's lower incisor dentin can be used as a human substitute.

Some examples of studies using bovine teeth as human substitutes are: Roulet et al. conducted in 1982 (221) where both bovine dentin and enamel were evaluated for SR after being polished with prophylaxis and polishing pastes; the study of Hanes et al. (222) conducted in 1991 comparing the morphology of radicular dentin after root planning and application of citric acid or tetracycline by using the bovine dentin present in the coronal half of the root; the study of Schmidlin et al. (210) conducted in 2002 investigating the tooth substance loss resulting from mechanic, sonic and ultrasonic root instrumentation on bovine central tooth roots where profilometric analysis, SEM analysis and liquid scintillation were used; the study of De Mendonça et al. (223) conducted in 2008 where bovine root dentin surfaces were used for comparing the effects of Er:YAG lasers, an ultrasonic system and a manual curette by SEM and profilometric analysis; and the study of Sisera et al. (224) conducted in 2009 using bovine dentin blocks to evaluate the edge retention technology of curettes after extended instrumentation *in vitro*.

Also, in order to mimic the ceramic-dentinal marginal region; bovine tooth samples were used by Anami et al. (199) in 2012 and Pereira et al. (200) in 2016. In these studies, the effect of various cement removal techniques on SR and bacterial adhesion were investigated. In our study a similar tooth-restoration interface model was prepared based on these aforementioned studies.

In our present study, to achieve standardization and avoid dimensional changes, samples were scanned with a CAD/CAM system and the margins of the ceramic restorations were designed with a software to eliminate the factor of human error or a lack of technique sensitivity. Dental impression material was not used since the precise replication of tissues with dental impression materials are dependent upon the amount and direction of the contraction of the material (225), the anatomy of the tissues from which the impression is taken (225) and shape of the impression trays (226). Dimensional stability depends on time (225), storage conditions (227) such as thermal changes (225) and measurements of the original model-cast (228).

During the preparation of CSs, the instructions of the manufacturer were carefully and closely followed. After the cementation of DSs to CSs; the excessive resin cement material was removed by using the "quarter technique" and a sickle scaler. Even though two different techniques are recommended in the instructions of the used dual cure resin cement, *(Variolink® Esthetic DC*), the additional flowchart describing the use of the quarter technique was preferred. The study of Periera et al. (200) has concluded that photoactivation prior to removal of the cement reduced SR values. This study has also concluded that if polishing was performed after cement removal, no difference was observed between the SR of samples regardless of the cement removal technique. Anami et al. (199) have shown that the cement removal technique did not influence the Ra significantly. Based on these studies, it can be stated that our choice of the quarter technique does not influence the outcome of our study.

CSs were glazed since glazing is the most routinely preferred finish for ceramic restorations by many clinicians. Studies conducted on glazed surfaces indicate that glazing may be considered to be an ideal finish for ceramic restorations. It has been shown that glazed surfaces were uniform and smooth (187,195). It was also shown that compared to various other finishes, glazed surfaces were also more biocompatible (194). Glazed surfaces were found to cause minimal amounts of irritation to tissues (196), provide ease of cleaning and esthetics (191). Compared to ground porcelain, glazed surfaces were again found to be smoother yet still slight defects were observed (186). It was also shown that if glazed surfaces were further polished, the Ra decreased as well as bacteria and glucan adhesion to the surfaces (53). In our study a decrease in Ra was also seen after polishing the glazed ceramic surfaces.

Even though there are many advantages of glazed surfaces, compared to polished unglazed ceramic surfaces, glazed surfaces were also found to contain large porosities causing a higher SR (183,189). The reason for porosity formation was stated to be either due to improper condensation of porcelain, incorrect powder/liquid mixing ratio or changes in firing time and temperature during the preparation of specimens (189) or the method used for glazing (183). Manipulation of ceramic materials and glazing requires a skilled laboratory technician since the fabrication process is highly technique sensitive. Minor faults in the

laboratory may result in clinically unacceptable surfaces to be formed (183,189). When the surface is glazed, it can become undulated and rough (53). Such a surface will not only be irregular but also will promote the adhesion of bacteria and other substances. In the present study, the lack of porosities and surface irregularities before instrumentation indicates that the glazing procedure of the CSs were conducted in a technic sensitive manner. When our samples were evaluated by SEM micrographs no porosity formation or structural surface irregularities were observed on the glazed surfaces before cementation, after cementation and after polishing.

In our study, sample blocks were polished with a diamond high gloss polisher before periodontal instrumentation in order to smoothen all the surfaces since a sickle scaler was used for removing the resin cement. Polishing the margin of the restoration at the end of cementation is also recommended, however this recommendation is neglected by many clinicians. Also, in the clinic, the necessity for refinishing a porcelain restoration may become inevitable due to various adjustments resulting in increased SR (53,189). The rough surfaces must be either be polished and/or re-glaze to achieve the desired surface finish (13,53). Studies also have shown that glazed surfaces may not always be the smoothest surfaces even if the glaze is not broken and polishing with a fine particle size abrasive is necessary for obtaining a smoother surface (185,229,230).

Pereira et al. (200) have concluded that polishing (with a silicone rubber tip) reduced SR in the marginal region of ceramic restorations significantly regardless of the cement removal technique used. In our study, polishing after cementation also reduced the SR significantly for all the surfaces of the restoration interface. In tooth surfaces the SR value (Ra:0,311 ± 0,052 μm) was found above the threshold SR value after cementation but decreased below it (Ra:0,135 ± 0,031 μm) after polishing. In a study where the effect of polishing was evaluated by Yoon, Noh and Park (193), a 3-step polishing kit was used after repeated instrumentation with an US on glass ceramic and a statistically significant increase in the SR (Ra:28,54 ± 9,64 μm increased to Ra:56,66 ± 9,12 μm) was observed. In our study we have only used the fine polisher of our kit of choice and observed a statistically significant decrease for CSs (Ra:0,317 ± 0,065 μm decreased to Ra:0,219 ± 0,055 μm). This difference

may be due to the motion patterns followed during application of the polishing system or the polishing kit itself. The surfaces in our study were polished by circular motions as instructed by the manufacturer, however Yoon, Noh and Park polished their samples in one direction.

After cementation and polishing, our 100 blocks were randomly divided into 5 different periodontal instrumentation groups as the TiN curette, US, LOD, AP-P and AP-S.

In order to determine the pressure application to be adopted during instrumentation with the US and the LOD, two studies were taken into consideration. Schimdlin et al. (210) compared a sonic prototype, a magnetostrictive US and a hand instrument *in vitro* on bovine incisor teeth. In this study, power-driven instruments were applied with a pressure of 40 grams and hand instrumentation was done with a pressure of 500 grams. Also, in the *in vitro* study of Kishida et al. (144) 40 grams of pressure was applied when working with power instruments and 500 grams were applied when working with hand instruments. This study compared instrumentation with a Gracey curette (control group), the LOD, and an US. In our present study, based on these two studies, 40 grams of pressure was applied with the hand instruments and 500 grams of pressure was applied for US and LOD following the education and calibration of the investigator (DFB).

Instrumentation time with the LOD and US was set to 1 minute in the present study based on the study of de Mendonça et al. (223). De Mendoça et al. also instrumented root surfaces of bovine tooth samples and in this study a surface area of 24 mm^2 was instrumented for 20 seconds by an US, hand instrument or Er:YAG laser. In our study the surface area of each block was approximately 72 mm^2. Since this surface area is 3 times the size of the surface area instrumented by de Mendoça et al., instrumentation time in our study was determined to be 60 seconds. Application of the AP devices was done for 20 seconds based on the study of Petersilka et al. (155).

Different power settings can be seen when different studies using various power instruments are evaluated (144,210). In the present study, the AP devices were connected to the turbine (dental unit) with an integrated coupling adapter. Power setting was not adjustable for these devices. For standardization and a fair comparison of different instruments, all the

power-driven instruments in our study were used at the maximum power settings. In the future, studies can be conducted where the effects of these instruments are compared when they work at different power settings.

The hand instrument, Double Gracey Anterior, used in our study is marketed as an instrument which does not require sharpening. It was our instrument of choice, since it provided standardization of the sharpness of the hand instrument between different samples without the requirement of re-sharpening. Even though its use on the margins is not recommended by the manufacturer, it should be noted that it in some situations it is inevitable for the practitioners to use hand instruments on the marginal regions of restorations. Also, to our knowledge, currently there is only one study available on our instrument of choice by Gürsoy et al. (84) where the profilometric evaluation of the root surface was conducted after instrumentation with TiN curette, stainless steel curette, piezoelectric US with a special tip for root surfaces and an Er:YAG laser. In this study it was concluded that the TiN curette caused the least amount of increase in SR. The results of our study are compatible with the findings of Gürsoy et al. (84): on ceramic surfaces TiN curette did not increase the Ra significantly; on tooth surfaces and resin cement surfaces, TiN curette caused a minimum amount of increase in the Ra compared to other instrumentation modalities used in our study.

In our study, a piezoelectric US was used, and the angulation was kept at minimum as recommended by the manufacturer. For the tooth surfaces and the ceramic surfaces, US was found to be causing the most amount of increase in Ra compared to LOD, TiN curette, AP-S and AP-P. On the resin cement surface, US was found to be in the third place for causing the most amount of change in the Ra amongst 5 different instrumentation modalities. However, for resin cement surfaces statistically significant difference was not found between the US and all the other tools used. On SEM microphotographs of samples instrumented with US, surface irregularities of various characters can be seen on dentin, ceramic and resin cement surfaces.

In the study of Yoon, Noh and Park (193) repeated instrumentation with the US on the ceramic surface was evaluated. An increase of Ra was reported. Even though instrumentation was repeated for 10 times in this study, the increase in the Ra was very high

(initial SR Ra:2,35 ± 1,05 μm, SR after scaling 28,54 ± 9,64 μm). These researchers used a reciprocal movement (forward and backward) during instrumentation. However, in our present study, an imbricate movement was made during instrumentation. Both the ceramic ingot and the glaze used in this study are identical to the ones used in our study. However, there is a big difference in the initial SR of the ceramic surfaces (in the present study the initial SR of CSs is Ra: 0,317 ± 0,065 μm). The difference may be resulting from the technique sensitivity of the glazing procedure. In our study, SEM micrographs of the representative CS display a uniform surface which is compatible with the lower Ra values measured.

Schmidlin et al. (210) compared a sonic instrument prototype, a magnetostrictive US and hand instrumentation. The application time was 2 minutes however measurements were taken every 30 seconds. When the measurements taken at 1 minute after the start of instrumentation were considered, US caused a higher difference in Ra compared to the hand instrument. In our study, instrumentation with the TiN curette was found to be causing a less pronounced increase in Ra for all surfaces as well.

There are some studies where the US was not found to be causing the most increase in Ra compared to other instruments (22,223) whereas our conclusion is that the use of US causes the highest increase in the Ra for dentin and ceramic surfaces. The difference in the instrumentation time, applied pressure, tip angulation-type or power setting may be the influencing factors behind this difference.

Another power-driven instrument tested in our study was the LOD. Even though the main marketing point of this device is being gentle and comfortable for the patients, it has been stated that the vibration mode of this system is similar to the vibration mode of USs (143). Majority of the studies comparing this system with conventional power-driven instruments have adopted different power settings for the LOD and the conventional system (29,32-34,146) during comparison and this may have influenced the results of these studies. The present study concluded that the LOD increased the mean SR of the ceramic surfaces, tooth surfaces and the resin cement surfaces significantly ($p<0.05$). When the

differences of Ra between the groups were compared, our findings have shown that in all groups the LOD produced a less pronounced SR change compared to the US. However, the differences between the US and the LOD were not statistically significant for neither the tooth surface, the ceramic surface nor the resin cement. When compared with the TiN curette, the LOD caused the Ra to increase more on all surfaces; however, the difference was found to be statistically significant ($p<0.05$) only on the tooth surface. The SEM microphotographs taken after instrumentation with the LOD revealed that this instrument changed the surface texture of all surfaces.

Kishida et al. (144) compared the SR caused by instrumentation with the LOD (with or without polishing fluid), US and Gracey curette. The US was found to produce rougher surfaces compared to the LOD and the Gracey curette. The average instrumentation time was not adjusted between the samples and was continued until the roots became smooth upon visual inspection and palpation with a probe. It was concluded that the mean time required to instrument a surface was higher when the LOD was used compared to the conventional US. Even though a shorter instrumentation time was reported, the increase in the SR caused by the US was found to be significantly more ($p<0.05$) compared to all other groups. In our study a statistically significant difference is present between the LOD and TiN curette for the change in mean Ra of tooth surfaces ($p<0.05$). The angulation of the tip of the instruments was not stated by Kishida et al. (144) and this may explain why a statistically significant difference was not found between the hand instrument and the LOD in this study when tooth surfaces were instrumented. Since it was shown that the TiN curette used in our present study increased the Ra less compared to stainless steel curettes (84) used by Kishida et al., the difference between the hand instrument and the LOD may have become more pronounced in our study. Also, the power was set to medium for both power-driven instruments in the study of Kishida et al. however in our study both instruments were set to high power setting. An increase in the power setting of LOD may have caused the instrument to have a more pronounced effect on the root surface and this may have also affected the result.

In our present study air polishing was conducted with a trehalose APP of two different grain sizes. Currently studies available on AP with the trehalose powder in the literature are very scarce. Chiesa et al. (231) compared the effectiveness of trehalose APPs on natural and

artificial dental surfaces in an *in vitro* study. Application was done at an angle of 70° for 5 seconds. The distance between the nozzle and the surface was 3mm. When working with the ≈65µm powder, our application angulation was ≈60° as recommended by the manufacturer and the working distance was set to be 4mm. The distance difference of 1mm may not be clinically relevant. Even though it is known that the angulation between the nozzle and the surface is an important factor (157) it has been stated that the short working distances in dental application may reduce or mask the effect (150). It has also been shown that the angulation of the instrument may not cause a noticeable difference in dental applications (155).

When the ≈65µm APP was used by Chiesa et al. (231), an observation of abrasion was reported but it was also stated that the SEM microphotograph was "difficult to evaluate". In our present study it was seen that the application of the ≈65µm trehalose powder increased the Ra of the tooth surface, the ceramic surface and the resin cement surface significantly ($p<0.05$). On the resin cement surface, it was found to be the tool causing the highest increase in Ra. Our resin cement surface SEM microphotographs revealed partially removed resin revealing a clear outline of the glass filler particles after application of AP-S using the ≈65µm APP. On tooth surfaces it increased the Ra significantly more ($p<0.05$) compared to TiN curette and AP-P. Our dentin SEM microphotographs after AP-S application revealed a rough but organized surface texture. On the ceramic surfaces, it increased Ra more than the TiN currete, the LOD and the AP-P Again, alterations in the surface texture of the ceramic sample can be seen on the SEM microphotographs.

Chiesa et al. (231) did not specify the use of a subgingival nozzle for working with the ≈30µm powder. In our study we have used the subgingival nozzle as recommended by the manufacturer and the tip was held parallel to the long axis of the sample without applying any lateral force. When SEM microphotographs were evaluated, Chiesa et al. (231) reported changes resembling "scratch-like grooves" on the surface when the fine grained powder was used on lithium disilicate ceramic. However, on our SEM microphotographs, no significant surface changes were observed for CS after instrumentation with the similar system and a statistically significant Ra increase was not recorded after profilometric evaluation.

In this present study when air polishing was conducted with the subgingival nozzle and the ≈30μm trehalose powder; the Ra of the ceramic surface did not increase significantly. However, a significant increase in the Ra was seen on the dentin and resin cement surfaces ($p<0.05$). When compared to other instrumentation methods in our study, the use AP-P was found to increase the Ra of the ceramic surface the least. On dentin surfaces, it caused the least increase in Ra second to TiN currete. The change caused by AP-P was statistically significantly less compared to LOD, US and AP-S ($p<0.05$). SEM microphotographs of dentin surfaces after AP-P application revealed no observable surface irregularities and the smear layer was removed revealing open tubule entrances. On the other hand, when the resin cement surfaces were evaluated, AP-P was found to cause the second highest increase in the Ra value, after AP-S. Since restoration margins are already susceptible to plaque accumulation (200), the use of the APs may be avoided at these sites since they increase the SR of the resin cement more than any other instrumentation method in our study based on our findings.

To conclude, the results of our study indicate that on tooth surfaces, the use of TiN curettes and an AP device with the perio nozzle and an APP of ≈*30* μm grain size may be the choice of instrument. On the other hand, compared to other instruments in this study, use of the LOD and the US has to be done carefully and avoided if only DPB has to be removed since it creates the most prominent changes in surface characteristics of dentin. On ceramic surfaces, the use of TiN curettes and an AP device with the perio nozzle and an APP of ≈*30* μm grain size may be the choice of instrument since their use does not increase the Ra or alter the surface topography significantly. On resin cement surfaces; using hand instruments (TiN curettes) may be preferred over the use of ultrasonic power-driven instruments and APs. For resin cement surfaces the use of AP devices may have the most abrasive effect regardless of the grain size of APP used and cause clinical problems in the long-term.

To our knowledge there aren't any previous studies conducted on the effects of various types of periodontal instrumentation conducted on a tooth- restoration margin model *in vitro*. There are also no studies available on the effects of periodontal instrumentation to resin cement surfaces. Further research can be conducted on how different instrumentation modalities affect the tooth-restoration interface and how these effects may have periodontal

consequences. Also, further research is required on how the SR of instrumented surfaces can be reduced by using various polishing systems after instrumentation since the present study shows that the surface characteristics of the interfaces of restorations are affected by periodontal instrumentation. The increase in SR and alterations of the surface morphology may predispose the margins to more DPB accumulation and make the surrounding soft tissues more susceptible to periodontal disease. A clinician should understand and educate themselves about the effects their instrument of choice has on the instrumented hard tissues in order to choose the most suitable method.

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