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AN IN-VITRO EVALUATION OF THE EFFICACY OF ORAL DEVICES TO REMOVE DENTAL BIOFILM FROM THREE PROSTHODONTIC MATERIALS

by

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DECLARATION

I hereby declare that "*An in-vitro evaluation of the efficacy of oral irrigating devices to remove dental biofilm from three prosthodontic materials*" is my personal work, that it has not been submitted previously in its entirety or in part for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged by a complete list of references.

Omnia Ahmed : 

Date Signed : 9 November 2018

DEDICATION

To my lovely parents who taught me to care for others, for teaching me the right way and to be a good human being. I dedicate my research to my parents who have supported me in every way possible and have shown me the value of education, perseverance and passion and whose love sustains my journey. I am a fortunate product of their dream.

To my brothers who supported me in this journey, encouraged me all the way and made this endeavor possible. To my brothers who continue to show me the road ahead. May Allah bless them all. To all my family and colleagues for their help and wishes for the successful completion of this research.

To my patients who entrusted me with their care. It has been a privilege and an honor.

The Prophet Muhammad (peace be upon him) said:

"One who treads a path in search of knowledge has his path to Paradise made easy by God"

ABSTRACT

Introduction: The evolution of Dentistry witnessed an increase in fixed prostheses as opposed to removable ones. Zirconia (ZrO_2) and Lithium disilicate (LDS) are becoming the material of choice in implant or tooth retained prostheses. Polyetheretherketone (PEEK) is a recent alternative as it is lighter and causes less wear of opposing retained teeth. Biofilm formation is a permanent daily struggle for patients as it can be found in nearly all surfaces exposed to the natural environment. Therefore, the interest in a new device capable of removing or reducing oral biofilm from fixed prostheses is increasing. Aquaflosser (AQ) and Waterpik (WP) are examples of these oral irrigating devices that were introduced to the dental market recently. They can be effective in removing dental biofilm from different surfaces.

Purpose of study: The purpose of this study is to evaluate biofilm formation on three fixed dental substructures and to evaluate the efficacy of two oral irrigating devices on biofilm removal from these three substructures.

Materials and Methods: A total of 60 samples were used in this study: 20 samples from each of the three tested materials namely Zirconium dioxide (Ivoclar Vivadent), Lithium disilicate (Ivoclar Vivadent) and Polyetheretherketone (White Peaks Dental Solutions). Scanning electron microscopy (SEM) was used to image the surface roughness of each sample because of its high resolution and magnification. Each sample was polished on one side and incubated with *Streptococcus mutans* (*S. mutans*) for 6 days. To evaluate biofilm formation on the three tested materials, the colour changes on each sample was determined using a spectrophotometer before and

after culturing of the *S. mutans* on the three materials. The mean values were calculated to determine biofilm formation on the three materials. Two different oral irrigating devices were used namely Aquaflosser (Home brand CC SA) at a pressure of 55 to 90 psi and Waterpik (INC.USA) at a pressure of 45 to 75 psi. The efficacy of the two pulsating devices to remove biofilm from the three materials was determined by measuring the colour changes of materials after biofilm formation with the materials after cleaning with the two pulsating devices. The mean values were calculated for each sample and compared.

Results: SEM results showed that the polished surface of PEEK exhibited the roughest surface, LDS recorded smoother polished surface, whereas ZrO_2 recorded the smoothest polished surface with relatively small pits and scratches. The biofilm formation showed a significant difference among the three tested materials (Tukey, $p<0.05$). PEEK showed the highest mean value of ΔE^* when compared with ZrO_2 and LDS, while ZrO_2 showed the lowest mean value. Although ZrO_2 showed the lowest mean value of ΔE^* there was no statistically significant difference compared to LDS. When the oral irrigating devices were compared, there was no statistically significant differences (Tukey, $p<0.05$) between Aquaflosser and Waterpik.

Conclusion: PEEK showed the highest biofilm formation compared with ZrO_2 and LDS. Although ZrO_2 had the lowest biofilm formation, there was no statistically significant difference ZrO_2 and LDS. Both Aquaflosser and Waterpik were efficient in the removal of the biofilm from the surfaces of the three prosthetic materials.

Keywords: biofilm, zirconium dioxide, lithium disilicate, polyetheretherketone, aquaflosser, waterpik

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AQ	Aquaflosser
ATCC	American Type Culture Collection
BF	Biofilm Formation
BHI	Brain Heart Infusion Broth
BL	Baseline
EDS	Energy Dispersive Spectroscopy
EPS	Extracellular Polymeric Substance
FDP	Fixed Dental Prosthesis
HIP	Hot Isostatically Pressed
LDS	Lithium Disilicate
Mcf	McFarland Standard
95% CI	95% Confidence Interval
PEEK	Polyetheretherketone
PIA	Polysaccharide Intercellular Adhesion
PIII	Plasma Immersion Ion Implantation
SEM	Scanning Electron Microscopy
SFE	Surface Free Energy
WP	Waterpik
ZrO_2	Zirconium Oxide

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Bacterial adhesion or biofilm formation is one of the principle aetiological factors of oral infections (Filoche *et al.,* 2010). Knowledge about bacterial adhesion and biofilm formation in restorative materials is therefore essential. Bacteria can form a biofilm on teeth and oral soft tissues and restorative materials. Biofilm formation and subsequent bacterial colonization are the main aetiological factors associated with the development of secondary caries, periodontitis and peri-implantitis.

Thus, restorative materials require properties that will assist in the prevention of bacterial adhesion and biofilm formation (Quirynen & Teughels, 2000). However, once formed this biofilm should be removed. A new technique to overcome the shortcomings of the commonly available techniques for removing dental biofilm from different restorative materials has recently been launched, known as an oral irrigating device. The principle of this technique is the removal of dental plaque with the aid of water pressure.

This review will focus on biofilm formation, prevention and reduction among three different materials, including zirconium dioxide (ZrO_2), polyetheretherkitone (PEEK) and lithium disilicate (LDS).

1.2 Dental Biofilm

According to Al Moaleem, *et al.,* (2017), dental biofilms are matrix-enclosed bacterial populations that adhere to surfaces such as polished tooth surfaces, living tissues, prosthetic devices and dental materials. Before discussing prevention of dental biofilm, it is important to describe what biofilm is. This concept is an expression used to describe a community of microbes cultivated on a surface, commonly with a liquid interface. Concerning mechanical properties, biofilm is referred to as a grouping of microbial cells associated with surfaces and which are usually enclosed in a polysaccharide material (Kokare *et al.,* 2009; Donlan, 2002). Biofilms where discovered by one of the researchers who used a primordial microscope to describe the accumulation of "animalcules" abraded from the human tooth surface (Costerton, 1999).

Its structure is composed of 80-85% extracellular polymeric substance (EPS) and 15-20% micro-colonies from different species including microbial cells 15-20% (Kokare *et al.,* 2009). The EPS is an essential component that is composed of polysaccharides (hetero-and homo-polysaccharides), proteins, and extracellular DNA and lipids, but it may vary in chemical and physical properties (Donlan, 2002). The EPS in biofilm has several positive effects.

The adhesion is the key function of EPS as it allows the initial steps in the colonization of abiotic and biotic surfaces by planktonic cell and long-term attachment of whole biofilms to surfaces (Gilan & Sivan, 2013; Karatan & Watnick, 2009; Vu *et al.*, 2009). Its function is to maintain the integrity of the biofilm and to limit the desiccation of the biofilm. It also prevents harmful substances such as antibiotics from getting through to their target (Donlan, 2002; Flemming & Wingender, 2001) by the formation of bacterial 'towers' surrounded by polysaccharides to reduce physical contact between

the antimicrobials and viable cells (Xiao *et al*., 2012; Hoyle & Costerton, 1991). The EPS has both hydrophilic and hydrophobic properties, because it can incorporate large quantities of water into the structure by hydrogen bonding (Karatan & Watnick, 2009; Donlan, 2002). Concerning the exchange of new genetic information, Karatan and Watnick (2009) emphasized that EPS facilitates horizontal gene transfer between biofilm cells. In microbial communities, the EPS also has protective effects because it provides resistance to nonspecific and specific host defenses during infection (Kokare *et al.*, 2009; Watnick & Kolter, 2000).

Plaque is a microbial biofilm composed of multiple colonies of bacterial species that populate on the oral cavity causing dental diseases (Marsh, 2005). Oral biofilms are well-organized communities of microorganisms surrounded by a polysaccharide-based matrix containing nucleic acids, proteins and H_2O that adhere to teeth, dental restorative structures or oral soft tissues (Teughels, 2006). Souza, *et al.,* (2016) found some factors that contributed to the growth and development of biofilms in the oral cavity. These included change in pH reaching low points after the intake of acidic substances and variance in temperature during the intake of warm or cold foods.

Furthermore, there is a variation of oxygen in the oral cavity, for example the low presence or absence of oxygen content in the areas below the gingival margin. Therefore, the microbial colonization in the mouth follows the variation of oxygen and pH, which promotes the preferential growth of aerobic or anaerobic microorganisms (Belibasakis *et al.*, 2015). A harmonious relationship exists between the microbiota of the oral cavity and host tissues in a healthy state. Thus, oral biofilms can be considered an integral part of the healthy mouth, as they can restrict the growth of pathogenic microbial strain, provide environmental stability over time, enhance epithelial barrier function and realign the host immune system (Kumar & Mason, 2015).

It also serves as a reservoir for fluoride (Naumova *et al.*, 2012). After all, an imbalance between microorganisms and host tissues can lead to oral diseases such as gingivitis and periodontitis (Lindhe *et al*., 2015).

1.3 Biofilm Formation in the Oral Cavity

Biofilm formation is a requirement for the existence of all microbial groups (Vu *et al.*, 2009; Flemming & Wingender, 2001). Studies on biofilm formation have largely been conducted to develop numerous surface modifications in order to reduce and prevent bacterial attachment to the surfaces (Bazaka, *et al.*, 2012.). Some scholars argued that there is no acceptable theory to explain the mechanism of bacterial adhesion and biofilm formation in the oral cavity (Bos *et al*., 1999; Hermansson, 1999).

Every microbial biofilm community is unique, although its structure can be the same (Flemming & Wingender, 2010; Flemming, *et al*., 2007). The biofilm formation can be controlled by various genetic and environmental factors (Marić & Vraneš, 2007). Busscher *et al.,* (2010) suggested that biofilm formation depends on the physio-chemical characteristics of the material's surface and is influenced mainly by the underlying biomaterial.

The salivary conditioning films play a significant role in biofilm formation due to the fact that they provide the main source of nutrients for microorganism adhesion and allow the coating of hard or soft surfaces with a thin (5-10 μm thickness) heterogeneous and acellular pellicle, known as pellicle or conditioning film (Souza, *et al*., 2016; Teughels, *et al*., 2006). The salivary proteins present in the oral environment are primary elements in the initial process of biofilm formation (Elter *et al*., 2008). However (Siqueira *et al*., 2012) emphasized that biotic and abiotic dental surfaces are constantly coated with saliva, which forms a conditioning film known as a pellicle,

consisting of glycoproteins (mucins), phosphoproteins, histidine-rich proteins, proline-rich proteins, α-amylase, and many other molecules, including bacterially derived glucosyltransferases (Siqueira *et al.*, 2012). Pellicle can be formed on any surfaces (Aroonsang *et al.*, 2014), although the exact effects of material properties on pellicle formation and the underlying mechanisms are not well understood. Researchers concluded that biofilm formation was a dynamic process involving various phases.

Souza *et al.*, (2016) categorized biofilm formation into four distinct stages including acquired pellicle formation, primary colonization, secondary colonization, and mature biofilm establishment. Garett *et al.*, (2008) defined the gathering of bacteria on a surface as a mechanism consisting of three stages: firstly, the organism is accumulated on a surface (collector) which is called adsorption. Secondly, the formation of a polymer bridges between the organism and collector by means of the attachment of the organism to the collector. Thirdly, the colonization or growth and splitting of the organism on the collector's surface. (Characklis & Marshall, 1990) divided bacterial accumulation into eight steps that include the formation of an initial conditioning layer, reversible and irreversible adhesion of bacteria, and the detachment of cells from a mature biofilm for subsequent colonization.

The conditioning layer possesses organic and inorganic particles and is considered as the main layer for biofilm growth. This layer alters the surface allowing bacterial penetration. The surface provides a harbour and nutrients for the growth of the bacterial community.

The reversible adhesion includes transportation of the microbial cells to the conditioned surface by means of physical forces or bacterial projections such as flagella. A fragment of the cells extending to the surface will reversibly be absorbed. Many local

environmental factors, such as temperature and pressure conditions, can affect bacterial adhesion (Khelissa *et al.*, 2017; Moncmanová, 2007). Physical forces which include Van der Waal's forces, steric interactions and electrostatic (double layer) interaction that are collectively known as DVLO (Derjaguin, Verwey, Landau and Overbeek) forces, also affect bacterial adhesion (Garett *et al.*, 2008). The DVLO theory describes a balance between two factors, Van der Waal's interactions (attractive) and the repulsive interactions from the overlap between the electrical double layer of the cell and the surface. If the repulsive forces are more than the attractive forces, the bacteria will dissociate from the surface (Chang & Chang, 2002).

When the reversibly adsorbed cells remain inactive, irreversible adhesion occurs, in which the physical projections of bacteria overwhelm the physical repulsive forces of the electrical double bond (Garett *et al.*, 2008). Consequently, chemical reactions such as oxidation and hydration will be stimulated from the contact between the bacterial projections and the surface (Kumar & Anand, 1998).

The immobile cells will then dissociate by binary division and the daughter cells will proliferate and form clusters (Hall-Stoodley & Stoodley, 2002). The clusters appear in a mushroom-like structure allowing the passage of nutrients to bacteria deep within a biofilm. Exponential growth occurs from the rapid increase in the population. At this stage, the bonding between cells becomes stronger due to the interaction between divalent cation and the polysaccharide intercellular adhesion (PIA) polymers. A number of expression genes were found to play a major role in bacterial attachment and proliferation, for example, the production of surface projections is constrained in sessile species as motility is restricted, thus expression of a number of genes for the production of cell surface proteins and excretion products increases (Garrett *et al.*, 2008).

In the final stage of biofilm development, a series of cell signaling mechanisms occur which are collectively termed quorum sensing (Bassler, 1999). Quorum sensing is a procedure in which a number of auto inducers (chemicals and peptide signals in high concentrations) are used to motivate genetic expression of both mechanical and enzymatic processors, which form the principle part of the extracellular matrix (Marsh & Zahra, 2017). The disruption of the polysaccharides holding the biofilm together occurs through the production of enzymes from the community itself resulting in the release of surface bacteria for the colonization of a fresh surface.

Hyaluronidase by *Streptococcus equi* and alginate lyase by *Pseudomonas aeruginosa* are examples of the enzymes responsible for the disruption of the biofilm matrix (Garrett *et al.*, 2008). At the same time, the organisms have the apparatus for mobility by the up regulation of the operons coding for flagella proteins and down regulation of the genes coding for porins (Marsh & Zaura, 2017).

In 2002, Donaln classified biofilm formation into four stages: Initially, the bacteria will be attached to the biological surfaces (Donaln, 2002). Several organic molecules including nutrients, salivary proteins and large macromolecules play a fundamental role in bacterial attachment to the surface (Larsen & Fiehn, 2017). This attachment is implemented with the aid of Van der Waal's forces between the cell surface and the substratum, which results in a strong adhesion-receptor, mediated attachment.

Irreversible interaction is facilitated by flagella, fimbriae and exopolysaccharides that are found in the bacterial cell wall. Secondly, the first attached cells start growing and dividing, leading to the up growth of micro-colonies (primary colonizers). The micro-colonies cohere together forming a layer of cells externally. The tertiary step is indicated when several layers of cells aggregate on the surface, forming cultured

biofilm characterized by the existence of large communities of bacteria surrounded by water channels that assist in distributing minerals and signaling molecules. Finally, some biofilm can disassociate individually or in clusters in order to diffuse and migrate to other niches. Biofilm distribution depends on the conditions that promote its growth as a result of environmental changes (O'Toole *et al.*, 2000).

Hannig and Hannig (2009) reported the forces responsible for bacterial adhesion and surface assimilation of selective salivary proteins. These forces include Van der Waal's forces, electrostatic reciprocal action and acid-based bonding. Derjaguin-Landau-Verweij and Overbeek developed the DLVO theory that stated that these forces can be joined and the energy calculated (Van Oss, 1995).

Regarding bacterial attachment to different surfaces, Glantz (1969) concluded that bacteria can attach to every surface and is not affected by its properties. This was confirmed by Boks *et al.*, (2009) who reported that hydrophobic surfaces return less biofilm than the hydrophilic ones. This occurs as a result of forces in the mouth, but in the test tube, bacteria can also attach to hydrophobic surfaces. Regarding the viability of biofilms, Auschill *et al.*, (2002) found that thin biofilms are more viable than thick ones due to a hampered supply of nutrients on a thick biofilm.

1.4 Factors Affecting Biofilm Formation

Both physical and chemical properties of a dental material as well as environmental factors can influence biofilm formation. These include surface roughness, surface charge, surface chemistry, topography; and hydrophobicity (Gilan & Sivan, 2013). However, scholars have demonstrated that surface roughness was an important physical factor in biofilm formation for various reasons (Anselme *et al*., 2010). For example, it increases bacterial attachment in the contact and surface area between the material

surface and bacterial cells. Ammar *et al.*, (2015) investigated the influence of surface roughness in biofilm formation. Their findings suggested that three parameters could be linked to surface roughness, which include: asperity mean size, roughness (Root Mean Square RMS) asperity radius and surface coverage. All these parameters have an impact on the height of the energy barrier (Ammar *et al.*, 2015). Similarly, the roughness acts as a barrier to shear forces and thus cleaning becomes difficult (Truong, *et al*., 2009; Bos *et al.*, 1999). Teughels, *et al.*, (2006) evaluated the effect of surface characteristics on biofilm formation. When integrating surface roughness with other characteristics (free energy and chemistry), their results revealed that surface roughness was the most critical factor.

Furthermore, Ionescu *et al.*, (2012) highlighted that smoothening of the surface had also been considered to reduce biofilm formation, and a Ra roughness of 0.2 µm was reported to be the threshold for maximum reduction of bacterial adhesion on abutment surfaces (Quirynen *et al*., 1996). However, the exact effects of surface roughness on bacterial adhesion and biofilm formation vary with the size and shape of bacterial cells and other environmental factors. Thus, there is no universally optimum roughness that can repress adhesion of all bacterial species (Renner & Weibel, 2011).

Empirical evidence showed that surface charge could play a major role in determining the binding force between bacteria and surface, affecting the biofilm formation (Song, *et al.*, 2015). As an overall net charge carrying the cell, the surface charge of a bacterial cell could influence the biofilm formation due to its excess carboxyl and phosphate groups, which are located in the cell walls of bacterial cells (Goulter, *et al.*, 2009). Most bacterial cells are negatively charged, thus, a positively charged surface is more prone to bacterial adhesion and a negatively charged surface is more resistant to bacterial adhesion (Ukuku & Fett, 2002).

Simultaneously, surfaces with cationic groups, such as quaternary ammonium have antimicrobial activities and thus can kill the attached bacterial cells (Campoccia *et al*., 2013). However, controlling bacterial adhesion with surface charge may not work in static systems since the dead cells present a barrier that reduces the charge and facilitates the adhesion of other bacterial cells (Foong *et al.*, 2017). Since shear force (rinsing and brushing) can be readily applied to remove dead cells from dental materials, this strategy may be effective in some oral applications.

In addition, surface free energy can influence bacterial adhesion, but generally, there is a difference between supra-gingival and subgingival surfaces. Supragingivally, less biofilm is formed on hydrophobic surfaces than hydrophilic ones, unlike subgingival biofilms where no differences are observed (Foong *et al.*, 2017). This difference was attributed to the fluctuating shear force in the supra-gingival environment, which can slough off bacterial biofilms more effectively from hydrophobic surfaces (Quirynen *et al.*, 1995). The preference of surface hydrophobicity differs among the bacterial species. The presence of salivary coating on bacterial cells and dental surfaces can also greatly affect how hydrophobicity influences the interaction between oral bacteria and dental materials (Foong *et al.*, 2017).

Studies compared biofilm formation with various types of surfaces indicating multiple factors involved such as roughness, and electrostatic interactions (De Avila *et al.*, 2014; van Brakel, *et al*., 2011). However, when comparing the effects of surface charge, hydrophobicity, chemistry, and the manner in which surface stiffness and topography (except for roughness) affect bacterial adhesion, biofilm development is still poorly understood (De Avila *et al.*, 2014). Almost all reviews showed that polished surfaces of the restorative materials harvest fewer biofilms compared to unpolished surfaces (Teughels *et al.*, 2006; Verran & Maryan, 1997).

Teughels *et al.,* (2006) confirmed that the adhesion of biofilm to restorative materials is aided by a rise in surface irregularity over 0.2 μm or a rise in the size of the surface. Quirynen and Bollen (1995) concluded that the roughness of intra oral surfaces (i.e. teeth, mucosa) has a large influence on the first attachment and accumulation of microbes and subgingival roughness will aid in the accumulation of more microbes. Wise and Dykema (1975) showed the importance of surface free energy and roughness on biofilm adhesion and highlighted that the surfaces should be as smooth as possible to minimize such adhesion.

Flemming *et al.,* (2016) confirmed that the chemical composition of a surface has a direct effect on bacterial growth and biofilm formation as it consists of beneficial or harmful components. For instance, a metal, such as brass, has bactericidal properties because it contains copper. On the contrary, polyvinyl chloride contains carbon and hydrogen that assist in biofilm formation (Flemming *et al.*, 2016)

Stoodley *et al.,* (1999) reported that the flow rate of saliva had a direct impact on bacterial adhesion and biofilm formation. Laminar flow rate (low shear) causes irregular micro-colonies to develop which consist of circular cell clusters separated from each other by interstitial voids, whereas biofilms that grow in a turbulent flow rate appear as patches of swelling and elongated streamers that vibrate in the flow (Stoodley *et al.*, 1999).

It is well known that the environmental conditions which influence biofilm formation include the effect of pH, adhesive properties of biofilms and the effect of temperature. (Pagán, & Gonzalo, 2015) concluded that changes in pH have a strong effect on bacterial growth. The trans-membrane electrochemical gradient (proton motor force) generated by the extrusion of protons from the cytoplasm causes a passive influx of

protons that can be an issue for the cells seeking to regulate their cytoplasmic pH (Booth, 1985). Large differences in external pH can overcome this mechanism and can kill the microorganisms (Booth, 1985).

Bacteria can be affected by both external and internal changes in pH (Garett *et al.*, 2008). Many reviews demonstrate that a gradual increase in acidity increases the possibility of cell survival in comparison to a fast increase by the addition of HCL (Li, 2001). This explains that bacteria contain mechanisms to adjust to the small environmental changes in pH. On the other hand, there are cellular processes that do not adjust to pH changes such as exopolymeric substances (polysaccharides). The optimum pH for most bacteria is around 7, but it differs between species (Garett *et al.*, 2008).

Biofilms show both irreversible viscous deformation as well as reversible elastic recoil (Ohashi & Harada, 2004). Biofilm adherence is influenced by the viscosity of the polysaccharides that result when the matrix of the extracellular polymeric substances (EPS) reacts to stress by showing properties such as elastic tension and alignment of the polymer in the shear direction. These properties can be altered by increasing the temperature of the polysaccharides which results in the generation of a gel like substance that slowly increases in strength until a crucial point. At this point, the gel forms a solution (Klapper *et al.*, 2002).

Many studies concluded that for biofilm formation to occur, the optimum temperature for a microorganism is related to an increase in its nutrient intake that in turn relies on the presence of enzymes (Garett *et al.*, 2008). Thus, temperature corresponds to the reaction rate of enzymes and the development of cells. Environmental temperature also influences the bacterial adhesive properties (Garett *et al.*, 2008). Fletcher (1977) found

that a decrease in temperature will reduce both the bacterial surface polymer and surface area. Moreover, temperature plays a major role in the presence of bacterial surface projections and lowering the temperature will increase bacterial adhesion to the surfaces (Garett *et al.*, 2008). In addition, lowering the temperature will affect the arrangement of microbial polysaccharides (Garett *et al.*, 2008). Nisbet (1984) reported that at a high temperature microbial polysaccharides are arranged in a disordered state, while at a low temperature they are arranged in an ordered state that favors bacterial adhesion (Nisbet, 1984). On the other hand, Marion-Ferey *et al.*, (2002) noticed that the higher temperatures were not effective for biofilm removal due to the "baking effects", thus increasing the adherent of the biofilm to the surface (Marion-Ferey *et al.*, 2003)

1.5 Biofilm Formation at Different Extra-Coronal Restorations

Biofilm formation on biomaterial surfaces may significantly affect the behaviour or survival of the biomaterial itself (Astasov-Frauenhoffer *et al.*, 2018). Numerous materials are available on the dental market today that can be used for extra-coronal restoration. These materials include metal, ceramics, metal-ceramics and resin composites. Among these materials, zirconium dioxide (ZrO_2), polyetheretherketone (PEEK) and lithium disilicate (LDS) have recently become of great interest as a result of their superior physical and mechanical properties.

1.5.1 Zirconium Oxide (ZrO_2)

In its pure form, zirconium oxide (ZrO_2) is a polymorphic material generally derived from zircon (ZrSiO4) and baddeleyite (Vasylkiv & Sakka, 2001). It constitutes three crystalline forms depending on diverse temperatures, such as monoclinic ZrO_2 (m-ZrO_2), tetragonal ZrO_2 (t- ZrO_2) and cubic ZrO_2 (c- ZrO_2) (Vasylkiv & Sakka, 2001; Gauna, *et al.*, 2015). At the time of firing, zirconium oxide can be altered from one crystalline state to another. At firing temperature zirconia is tetragonal and at room

temperature monoclinic. Crack propagation can be created by high compressive stresses that result from the transformation of a tetragonal to a monoclinic phase, which occurs below 1170°C and is accompanied by a 3% to 5% volume expansion (Santos *et al*., 2015). To stabilize the tetragonal phase at room temperature to control the volume expansion, stabilizing oxides such as yttrium-oxide are added in small quantities to pure zirconia (Santos, *et al.*, 2015).

Yttria-stabilized tetragonal zirconia polycrystalline (Y-TZP) is a high-strength ceramic, recommended for dental use as a core or framework material for fixed dental prosthesis (FDP) and crowns (Denry & Holloway, 2010). The restorations are prepared either by soft machining of pre-sintered blocks followed by sintering at high temperature or by hard machining of fully sintered blocks (Denry & Kelly, 2008). Restorations manufactured by hard machining of fully sintered 3Y-TP blocks consist of a significant amount of monoclinic zirconia (Denry & Kelly, 2008). This usually results in surface micro-cracking, higher susceptibility to low temperature degradation and lower reliability (Hauang, 2003).

The mechanical properties of 3Y-TZP rely on its grain size (Kim & Ahn, 2013). Beyond a critical grain size, 3Y-TZP is less stable and more prone to t-m transformations, despite the fact that smaller grain sizes (<1 μm) are associated with a lower transformation rate (Cionca *et al.*, 2017). Below an absolute grain size (~2 μm) transformation is not achievable, leading to reduced fracture toughness (Cionca *et al.*, 2017). Therefore, the sintering conditions have a strong influence on both stability and mechanical properties of the final product (Denry & Holloway, 2010).

The Zirconia ceramics have a dense, mono-crystalline homogeneity and possess low thermal conductivity, low corrosion potential, good radiopacity, high biocompatibility,

low bacterial surface adhesion, and favorable optical properties (Anusavice *et al.*, 2013). Zirconia has double the flexural strength between (900 MPa to 1200 MPa) when compared with alumina ceramics (Anusavice *et al.,* 2013). Possible problems with Zirconia ceramics include long-term instability in the presence of water, veneering porcelain compatibility issues, aesthetic limitations due to their opacity, and no adequate bond with resin-based luting cements (Kelly & Benetti, 2011). Dental zirconia has properties that can be used in single- and multiple-unit anterior and posterior fixed dental prosthesis (FDP).

Previous reports showed that the most common clinical problems were not associated with cracking of the zirconia framework, but with chipping of the veneering porcelain (Miura *et al.*, 2015). These deficiencies may be associated with non-anatomic framework designs or with poor bonding between zirconia and veneer, while other theories suggest problems related to the material itself that are often associated with low degradation phenomenon at mouth temperature, auto-catalytic transformation during porcelain firing and residual stresses resultant from thermo-mechanical parameters (Heintze & Rousson, 2010).

1.5.2 Polyetheretherketone (PEEK)

Several new polymeric materials have recently been used as biomaterials in orthopedics and dental applications, which include polyetheretherketone. PEEK is a new synthetic, tooth coloured polymeric material that has been used as a biomaterial for dental applications (Pokorný, *et al.*, 2010) and was developed by a group of English scientists (Ortega-Martínez, *et al.*, 2017). It is formed by the polymerization of an etheretherketone monomer via step-growth dialkylation reaction. It is white in colour and has unique physical and mechanical properties allowing it to be widely used for dental applications including implants, fixed and removable prostheses and as an

aesthetic orthodontic wire (Schimidlin *et al.*, 2010). PEEK can be altered by addition of other materials such as carbon fibers, which increases the elastic modulus up to 18 GPa (Najeeb *el al.*, 2016). Due to its excellent mechanical properties, it is considered as an applicable biomaterial to replace metals, alloys and ceramics in the field of dentistry (Schwitalla *et al.*, 2015).

The material has been used by orthopedic surgeons for hip replacements and due to the fact that it is bio-inert and the modulus of elasticity is close to bone, the results look promising (Nakahara et al., 2012).

Regarding PEEK applications in dentistry, evidence suggested that PEEK could be a viable alternative to titanium in constructing implant abutments due to the fact that PEEK has a closer elastic moduli to human bone which decreases the stress shielding effects and encourages bone remodeling (Najeeb *et al*., 2016). Moreover, PEEK can be used for the fabrication of crowns. Multiple procedures have been implemented to condition the PEEK surface to make it easier for bonding with resin composite crowns (Tannous *et al*., 2012). These procedures include: air abrasion with or without silica coating which creates a more wettable surface (Stawarczyk *et al.,* 2013) and etching with sulfuric acid leading to the creation of a rough and chemically altered surface which may enable it to bond more effectively with hydrophobic resin composites (shear bond strength: 19.0 ±3.4 MPa) (Schmidlin *et al.*, 2010).

On the other hand, etching with piranha acid and the use of bonding agent has been indicated to produce tensile bond strength to composite resin as high as 23.4 ± 9.9 MPa in preserved, aged PEEK specimens (Najeeb *et al.*, 2016). Mohammadi *et al.*, (2017) further showed that no obvious variations were observed between the tensile bond strength of PEEK crowns and dentine abutments when using air absorption and

sulfuring acid etching techniques. Evidence suggested that the three-unit PEEK fixed partial dentures manufactured by CAD-CAM, have a higher fracture resistance when compared to pressed granular or pellet shaped PEEK dentures (Stawarczyk *et al.*, 2015). The fracture resistance of CAD-CAM milled PEEK fixed dentures is also much higher (2055 N) than those of lithium disilicate glass-ceramic (950 N), alumina (851 N) and zirconia (981-1331 N) (Kolbeck *et al.*, 2008).

PEEK abrasive properties are excellent, despite the low elastic modulus and hardness and PEEK abrasive resistance is on par with metallic alloys (Zok *et al.*, 2007). However, no clinical studies have been conducted to compare the abrasion on teeth produced by PEEK crowns to abrasion produced by other materials and therefore it is still unknown whether PEEK crowns can function in harmony with dentine and enamel.

1.5.3 Lithium Disilicate (LDS)

Lithium-disilicate (LDS) was first introduced by Ivoclar Vivadent as IPS impress and was the second generation of heat pressed ceramics that contains lithium disilicate as the main crystalline phase (Denry & Holloway, 2010). The ceramic microstructure consists of highly interlocked lithium-disilicate crystals, 5 µm in length and 0.8 µm in diameter (Denry & Holloway, 2010). It is indicated for crowns, veneers, inlays and onlays. It can be used for up to a three unit dental prosthesis in the anterior region (Toksavul & Toman, 2007). IPS Impress 2 showed a high clinical success rate when used as a single unit and a lower success rate for a three-unit prosthesis (Marquardt & Strub, 2006). IPS Impress 2 was discontinued and replaced by a newly formulated and reinforced lithium disilicate under the trade name of IPS e.max (Ivoclar, Vivadent). The manufacturer produces a pressable version (IPS e.max Press) and a partially crystallized ceramic for CAD/CAM machines (IPS e.max CAD) (Pieger *et al.*, 2014).

IPS Empress 2 has enhanced flexural strength (360 MPa) that is more than double that of leucite-based IPS Empress, suggested to be used for anterior and premolar crowns, as well as three-unit fixed dental prosthesis (FDP) in the anterior region (Oh *et al*., 2000). In order to produce an esthetic restoration with enhanced light transmission, the framework is veneered with fluorapatite-based veneering porcelain (IPS Eris, Ivoclar Vivadent), which has the same optical properties and co-efficient of thermal expansion as the lithium-disilicate material (Conrad et al., 2007).

IPS e.max Press was shown to have higher physical properties (flexural strength 400 MPa) than the former IPS Empress (Kelly & Benetti, 2011). Additionally, it is composed of a lithium-disilicate glass ceramic, with fine crystal size, that displays improved physical properties and translucency obtained through a different firing process (Kelly & Benetti, 2011). The material presents with high translucency despite its high crystalline content due to the relatively low refractive index of the lithium-disilicate crystals (Kelly & Benetti, 2011). The uses of IPS e.max Press, pressable lithium-disilicate ceramic, include monolithic application for inlays, onlays, and posterior crowns or as a core material for crowns and three-unit FDPs in the anterior region (Giordano & Mclaren, 2010).

Currently machinable lithium-disilicate blocks (IPS e.max CAD) were introduced for use with CAD/CAM processing technology. These blocks are prepared using a two-stage crystallization process (Santos *et al.,* 2015). These blocks are indicated for anterior or posterior crowns, implant crowns, inlays, onlays, and veneers, as they present with flexural strength of 360 MPa (Santos *et al.,* 2015). Despite the fact that core ceramic fracture resistance is comparatively high, veneered prostheses have been known to be susceptible to chipping (Apel *et al.,* 2008).

Biofilm formation on different extra-coronal restorations was evaluated by several investigators who concluded that the proliferation and colonization of microorganisms were dependent on the surface properties (chemistry), surface topography (roughness) and surface-free energy (Hamdan *et al.*, 2006).

Carinci *et al.,* (2004) found that the level of toxic products produced by bacteria was greater on titanium compared to zirconium oxide when measured using nitric oxide synthase. They reported that zirconia could regulate expression of some genes, thus it can be considered as a self-regulatory material that can lead to the replacement of the extracellular matrix.

An *in-vitro* and *in-vivo* study by Kantorski *et al.,* (2009) examined the attachment of bacteria and biofilms on ceramics in comparison with other materials such as titanium and composite. There was decreased bacterial adhesion and biofilm production on ceramics compared with other materials. However, limited information is available on bacterial adhesion on different types of ceramics, thus more studies are required.

A trial done by Scarano *et al.,* (2004) in which zirconia discs were glued on a device and worn intra-orally for a day, elicited less plaque accumulation when compared with titanium. This is ascribed to the superficial structure of zirconia, mainly its electric conductivity (Scarano *et al.,* 2004). Meier *et al.,* (2008) and Hahnel *et al.,* (2009), observed bacterial adhesion to different types of ceramics and concluded that streptococcal species are the only species that attach to different types of ceramics with no aggregation of biofilms. Auschill *et al.,* (2002) calculated biofilm formation during 120 hours on different dental materials, including one-glass ceramic and reported thicknesses of 1 to 17 µm by using intraoral appliances plus heavy staining of the biofilms.

This finding was further confirmed by Bremer *et al*., (2011) who examined biofilm formation on five different ceramic materials including glass-ceramic, lithium disilicate, Y-TZP zirconia, hot isostatically pressed (HIP) Y-TZP, and HIP Y-TZP with 25% alumina. It was concluded that the lowest surface coating with biofilm thickness 1.9 µm was found with HIP Y-TZP ceramic and the highest value with lithium disilicate 12.6 µm. Zirconia showed the lowest plaque accumulation; thus, zirconia can be widely used in different dental applications (Bremer *et al*., 2011).

Scarano *et al.*, (2004) recorded that bacteria can cover approximately 12.1% of zirconia surfaces in contrast to 19.3% of titanium surfaces. Rimondini *et al.,* (2002) showed similar results in which Y-TZP accumulated less bacteria than titanium. Concerning bacterial adhesion to PEEK, Najeeb *el al.,* (2016) found that PEEK coated with TiO_2, treated with Plasma immersion ion implantation (PIII), demonstrated partial activity against *Staphylococcus aureus* and *Escherichia coli,* but its antibacterial activity against periodontal pathogens was not observed.

Moreover, Wang *et al.,* (2014) recorded the antimicrobial activity of PEEK nano-composite, although more studies are required to determine the use and handling of composites before these composites can be used for restorative treatment. Another group of scientists found that there is no difference in the bone resorption and soft tissue inflammation around PEEK and titanium abutments (Koutouzis *et al.*, 2011). Additionally, Hahnel *et al.,* (2014) compared biofilm formation on the surface of PEEK abutments with those abutments made with titanium, zirconia and polmethylmethacrylate. The results showed almost similar biofilm formation on the various materials. Laboratory studies demonstrated that biofilms can be reproduced on the surface of PEEK (Williams *et al*., 2011) and recently it was reported that the adhesion and proliferation of oral streptococci is similar on the surface of PEEK

compared to a conventional resin based composite (Kolbeck *et al.*, 2013). A study analyzing microbial issues in implants supplied with PEEK abutments identified similar microbial counts and levels of periodontal pathogens in the peri-abutment region of implants supplied with PEEK and titanium healing abutments (Volpe *et al.*, 2008).

Viitaniemi *et al.,* (2017) compared the bacterial adhesion and early colonization of *Streptococcus mutans* (*S. mutans)* on four different materials including, Lithium disilicate (LDS), fully stabilized zirconia, partially stabilized zirconia and dual curing cement. Results suggest that LDS had lower *S. mutans* adhesion than other materials examined, but the difference was not reflected in early biofilm formation.

Hussein, *et al.,* (2016) investigated bacterial adhesion on three materials including zirconia, lithium disilicate and gold crowns. Seventeen participants from different provinces underwent professional cleaning and were instructed not to brush their teeth for 72 hours. Swabs were then taken from gold, LDS and zirconia crown surfaces and cultured. It was found that less *Streptococcus Sanguineous* colonies adhered to zirconia crowns compared to LDS and gold crowns (Hussein, *et al.,* 2016)

1.6 Consequences of Biofilm Formation Around Extra-Coronal Restorations

Biofilm formation on different restorative materials in the oral cavity may have adverse side effects depending on the site and sequence. For example, periodontitis and gingivitis can develop in interdental areas that are difficult for the patient to clean (Löe, 1979). Dental implants and their implant components consist of biomaterial surfaces that may favor the growth and development of biofilm. These surfaces can provide suitable conditions for biofilm formation and therefore may be considered a potential reservoir for reinfection with oral opportunistic microorganisms. The presence of gaps after implant attachment, related to implant systems of two components, may act as a

trap for biofilm adhesion and maturation (do Nascimento *et al*., 2012). Biofilm attachment on dental implants and implant components may result in inflammation of soft and hard tissues, leading to severe inflammatory reactions that may cause pre-implantitis and implant loss (Jansen *et al*., 1997). Therefore, there is a need for devices that inhibit or remove biofilm formation.

1.7 Removal Techniques of Dental Biofilm from Solid Surfaces

Natural ingredients present in saliva are considered the primary defense system in biofilm inhibition (Marsh *et al*., 2016). These consist of lactoferrin which modifies the membrane permeability through distribution of lipopolysaccharides resulting in microbial killing (Tenovuo, 2002). Lysosome is an enzyme that plays a symbiotic action with lactoferrin (Tenovuo, 2002).

Several techniques and products are available to prevent biofilm formation. These include toothbrushes, rinses, floss and dentifrices (Barnes *et al.,* 2010). Two main methods have been developed to control biofilm formation on different surfaces (Lee *et al.*, 1995). The first is the use of chemicals to kill the bacteria in the biofilm to induce the natural sloughing of dead biofilm, thus cleaning the surface and preventing corrosion (Nemours, 1984). The second is the use of shear forces that overcome the tensile strength of the matrix material without damaging the material's surface, thus removing the matrix-enclosed bacterial micro-colonies from the surface (Larsen & Fiehn, 2017).

The application of chemicals is not effective, as most antimicrobial agents do not penetrate the biofilm, thus it is difficult to distribute enough agent to clean the surface. However, the physical removal of biofilm is very successful in cleaning the surfaces and removing the bacteria from the surface completely (Gorur *et al.*, 2009).

Mechanical plaque control plays a fundamental role in inhibiting bacterial adhesion and biofilm formation (Wilson *et al.,* 1987). The most popular technique for mechanical plaque removal is tooth brushing. However, it does not reach the interproximal areas as effectively as the facial and lingual surfaces. Many other interdental cleaning aids have been developed but seem not to provide patient satisfaction (Saxer *et al.,* 1998).

Oral irrigating devices were introduced and considered as delivery devices for antimicrobial solutions. They remove plaque and food debris through a jet stream of water (Warren & Chater, 1996). Their mechanism of action consists of two constituents: pulsation and pressure. This blending allows for compression and decompression to assist the removal of subgingival bacteria and other debris (Jahn, 2010). Pulsating devices have also been found to be three times more effective than continues stream devices (Jahn, 2010).

Aquaflosser (Home Brand CC SA) and Waterpik (Inc, USA) are examples of these devices that were recently introduced on the dental market. They have the ability to remove dental biofilm from different surfaces. According to the manufacturer's instructions, it works by spraying and pumping water that can be used routinely for flossing and in more difficult cases for cleaning of implants, orthodontic appliances, crowns and bridges, ensuring healthier, cleaner teeth and gum.

They use a high-pressure spray of water and can therefore reach areas which cannot be reached by other devices. They also have the ability to remove food debris that sticks between the teeth. The low-pressure spray is used for gums that are more sensitive. Water pressure has been found to have a direct effect in biofilm removal. Kato *et al.,*. (2012) reported that most plaque biofilm could be irrigated by using vertical water pressure more than 350 Kpa. Although these findings suggest that plaque biofilm could

be removed when using a high power output, it was found that the use of high pressure solely was inadequate to remove plaque biofilm completely from the surface and thus there is a need for other aids.

Many investigators reported that the efficacy of oral irrigating devices is related to the mechanism of action *versus* the type of agents used (Pistorius *et al.*, 2003). Flemmig *et al.*, (1995) compared a dental water jet with 0.06% chlorhexidine, a dental water jet using water only and 0.12% chlorhexidine rinsing (all used once daily) with tooth brushing alone. They concluded that a dental water jet with chlorhexidine provided the best results for reducing plaque, bleeding and gingivitis, whereas a dental water jet with water only was better than chlorhexidine rinsing at reducing marginal bleeding and bleeding on probing.

Regarding shear forces, Gorur *et al.*, (2009) reported that a three second exposure to shear forces produced by a dental water jet with known pulsations per minute removed biofilm both over and beneath the cemento-enamel junction with 99.99% efficiency when using a jet tip and 99.84% efficiency when using an orthodontic tip.

Many cohort studies demonstrated the efficacy of a dental water jet in reducing bleeding and gingivitis when used with 1,200 pulsations per minute and a pressure of 55-90psi. Other clinical studies showed that patients with varying needs (e.g. patients with orthodontic appliances, implants, crowns and bridges) benefitted from the use of a dental water jet (Barnes *et al.*, 2005).

Brady *et al.*, (1973) examined the effect of a pulsating water jet, at a pressure setting of 70 psi, in removing supra-gingival biofilm on teeth of rhesus monkeys using an electron microscope. The results showed that the sites treated with the water jet showed

removal of biofilm or irreversible damage to the bacteria in the biofilm matrix compared to the untreated sites.

There are conflicting studies on plaque removal using a dental water jet, describing both positive (Barnes *et al.*, 2005) and negative results (Chaves *et al.,* 1994). A systematic analysis by Husseini *et al.* (2008) found that an oral irrigator does not induce useful results by reducing visible plaque. The above studies used plaque-scoring methods, which include the Carter and Barnes bleeding index, Löe and Silness Gingival index and Proximal/Marginal index. McCracken *et al.* (2006) concluded that plaque indices are considered the most important determinants of outcomes in clinical trials for any method of plaque removal.

The most common tip used on oral irrigating devices is the standard jet tip. The depth of penetration of the jet tip depends on the pocket depth and tip placement (Boyd & Baumrind, 1992). Burch *et al.*, (1994) observed the efficacy of oral irrigator devices in removing plaque from molar areas, mainly in cases of fixed prosthesis when compared with other interdental devices. The use of dental water jets reduced pro-inflammatory mediators (e.g. IL-1B and PGE2) and promoted the removal of salivary plaque biofilm independent of the water jet used (Al-Mubarak *et al.*, 2002).

1.8 Efficacy of Oral Irrigating Devices- Methods for Evaluation of Oral Biofilm Removal

Considerable research efforts are currently being implemented for developing methods and instrumentation to remove biofilms from material surfaces. Many different methods have been used to assess biofilm removal efficiency. Biological approaches include semi-quantitative staining, measurements of dried biomass, protein or DNA quantification, and assessments of residual viable organisms through standard microbial culture techniques (Hadi *et al.*, 2010). Each method has advantages and

disadvantages, but they all provide only indirect values of the removal efficiency and are prone to operator-induced variability.

Direct demonstrations of biofilm removal by microscopic methods are considered the most efficient techniques nowadays (Heersink *et al.*, 2003). This provides information on its structural characteristics, its interaction with the surface, as well as spatial information regarding the homogeneity of biofilm disruption. Confocal laser microscopy, electron microscopy, light microscopy, bioluminescence imaging and macro-scale photography have all been used as measurements to assess biofilm removal (Agarwal *et al.*, 2014; Zhang & Hu, 2013). These techniques are used qualitatively or semi-quantitatively leading to a high risk of bias in the investigation.

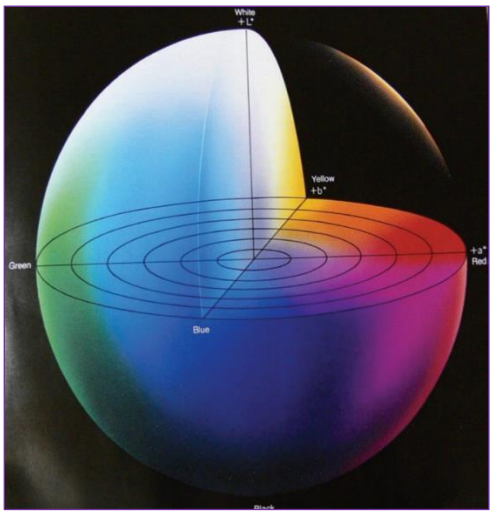
When quantitative measurements are made, information such as biofilm area/volume and thickness can be acquired to establish the effectiveness of the intervention (Nance *et al.*, 2013). Another method of biofilm removal is dividing an image into segments based on various image characteristics, known as segmentation (Frickle *et al.*, 2012). Segmentation has been used to calculate the area of the surface covered by biofilm. Manual thresholding has been used to segment images of biofilm from photographs and light microscopy images but this results in large errors and renders intra-operator reproducibility impossible (Frickle *et al.*, 2012).

Scanning electron microscopy (SEM) has been widely used in combination with biological assays of biofilm removal efficiency for qualitative observation of biofilm due to its high resolution (Li *et al.*, 2012). Using SEM images, simple thresholding cannot be performed, as the intensity values of the biofilm and normal surface are similar as seen on SEM. Rough surfaces can further complicate the image analysis and therefore advanced segmentation methods such as semi-supervised machine learning

techniques are used (Chan *et al.*, 2006).

Rimondini *et al.,* (2002) utilized a quantitative method to measure bacterial adhesion to ceramic materials *in vitro* using a spectrophotometer. The spectrophotometer is a device used to measure light intensity (photometer). It has been used to measure light absorption, but is now designed to measure spectral reflectance, transmission or relative emission by utilizing a high sensor that collects the reflected light from an object and processes it using a built-in microcomputer (Joiner, 2004, Guan *et al.*, 2005).

A spectrophotometer measures colour values in three different coordinates, L*, a* and b* (CIE L* a* b* colour parameters); where L* represents values in the lightness (black to white), a* represents values in the red-green coordinate, and b* represents values in the yellow-blue coordinate (Acar *et al*., 2016). All the values are absolute numbers with (1) the L* coordinate (range 0-100) measures the quantity of white-black: the greater the L value, the whiter the sample (0= black, 100 = white); (2) the a* coordinate measures the colour along the red-green axis: a high a* value refers to the amount of red in the sample, a low a* value refers to the amount of green; (3) the b* coordinate measures the colour along the yellow-blue axis: a high b* value is yellow, a low b* value is blue (Acar *et al*., 2016; Johnston, 2009) (Figure 1.1) is a visual representation of colour for the L*a*b* colour space.



(The L*a*b* colour space devised by the Commission Internationale d'lEclairage (International Commission on Illumination) includes all perceivable colours which may be described by coordinates in the sphere) (Maart et al, 2016)

Figure 1.1: A visual representation of colour for the L*a*b* colour space

CHAPTER 2

AIMS AND OBJECTIVES

2.1 Aims

The aim of this study was to evaluate biofilm formation on three fixed prosthetic materials namely zirconium dioxide (ZrO_2), lithium disilicate (LDS) and polyetheretherketone (PEEK), and to compare the cleaning efficacy of two irrigating devices on the biofilm removal from these three materials.

2.2 Objectives

- To measure the colour intensity of stained biofilm on ZrO_2, LDS and PEEK surfaces using a colour spectrophotometer on the L*, a* and b* scale.

- To measure the colour intensity of the biofilm after the application of the two pulsating water jet devices namely Aquaflosser (AQ) and Waterpik (WP)

- To compare the cleaning efficacy of these two cleaning devices.

2.3 Null Hypothesis

- There is no difference in biofilm accumulation of the three different materials tested namely ZrO_2, LDS and PEEK.

- There is no difference in the effect of Aquaflosser and Waterpik in removing

biofilm from ZrO_2, LDS and PEEK surfaces.

2.4 Ethical Considerations

The study was presented to the Dental Research and Senate Research Ethics committees of the University of the Western Cape for approval and permission to conduct the study (Ethics Reference Number BM 17/7/8). No human tissue was used in the study. Materials and oral devices were purchased from the manufacturers. There was no vested interest in any of the materials used. After the study, the materials were placed in a hazardous waste container for biomedical waste.

2.5 Conflict of Interest Statement

No conflict of interest is declared.

CHAPTER 3

MATERIALS AND METHODS

3.1. Study Design

This was an *in-vitro* study to evaluate and compare the efficacy of two oral irrigating devices regarding the ability to remove dental biofilm from the surfaces of three fixed prosthodontics materials.

3.2. Materials & Devices

3.2.1. Materials

Three types of materials were used in this study: zirconium dioxide (Figure 3.1), lithium disilicate (Figure 3.2) and polyetheretherketone (Figure 3.3) which were obtained from two different manufacturers (Table 3.1).

Table 3.1: Materials used in this study with their code, scientific name and supplied manufacturer

Code	Product name	Scientific name	Form	Manufacturer
Zirconia	Zenostar	Zirconium oxide	CAD/CAM	Ivoclar Vivadent, USA
LDS	IPS e.max	Lithium disilicate	CAD/CAM	Ivoclar Vivadent, USA
PEEK	Copra Peek	Polyetheretherkitone	CAD/ CAM monolithic	White Peaks Dental Solutions, Germany

Figure 3.1: Zirconium dioxide block as supplied by Ivoclar Vivadent, USA

Figure 3.2: Lithium disilicate block as supplied by Ivoclar Vivadent, USA

Figure 3.3: Polyetheretherketone block as supplied by White Peaks Dental Solution, Germany

3.2.2. Devices

Two types of oral irrigating devices were used in this study; Aquaflosser (Figure 3.4) and Waterpik (Figure 3.5). They were supplied from the manufacturers and used according to the manufacturer's instructions (Table 3.2).

Table 3.2: Oral irrigating devices used in this study with their commercial name, model, pressure and manufacturer

Commercial Name	Model	Manufacturer	Pressure
Aquaflosser	AQF 1000RE	Home brand CC SA	55 to 90 psi (517 Kpa) 1200 pulse per minute
Waterpik	WP-560	Waterpik INC. USA	45 to 75 psi(3.160 to 5.270 kg/cm2)

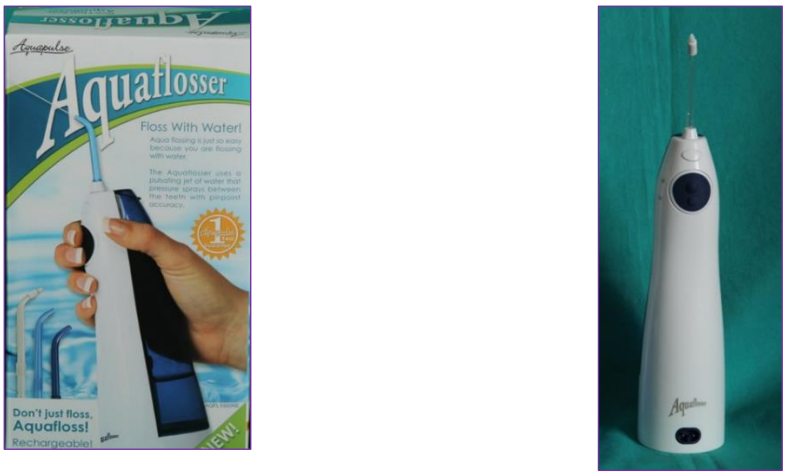


Figure 3.4: Aquaflosser with a pressure of 55 to 90 psi (517 kPa)

Figure 3.5: Waterpik with a pressure of 45 to 75 psi (3.160 to 5.270 kg/cm^2)

3.3. Methods

3.3.1. Sample Preparation

Twenty circular shaped discs (n=20) from each of the three tested materials (ZrO_2, LDS and PEEK) were prepared with a diameter of 18.5 mm and 4 mm in thickness (Figure 3.6).

Zirconium *Lithium disilicate* *Polyetheretherketone*

Figure 3.6: Sample discs - zirconium, lithium disilicate and polyetheretherketone

The CAD/CAM Sirona in Lab Dentsply (using software version 16.1) was used for the cutting of the samples (Figure 3.7). The samples were made in the software that then milled the blocks using Dentsply milling unit.

Figure 3.7: CAD/CAM Sirona in Lab (Dentsply) used for cutting of samples

A jig was constructed from polypropylene material (Figure 3.8) to fix the samples to in order to cut a hole at the periphery of each sample, so as to thread a piece of dental floss which then enabled it be suspended in a bottle.

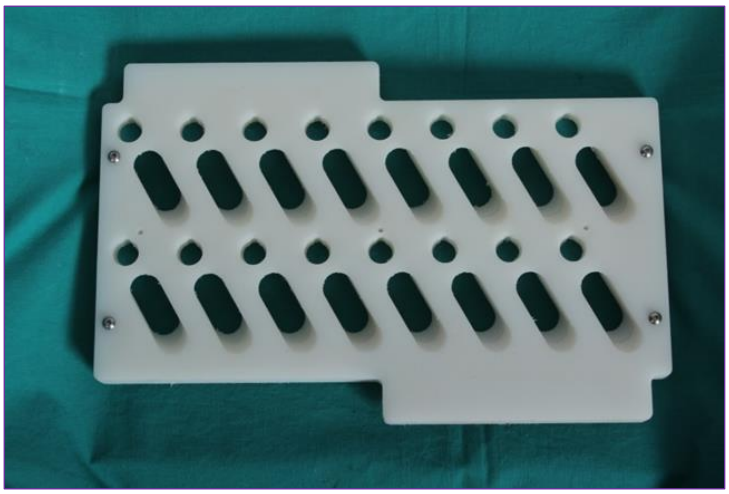


Figure 3.8: Jig constructed to facilitate cutting of the holes

The diameter of each hole was 1.05 mm and it is created to facilitate the holding of the material using dental floss (Figure 3.9).

Figure 3.9: Holes created at the periphery of each sample to be able to thread floss through it

The waterjet-cutting machine (Flow Waterjets, USA) was used for cutting of the holes (Figure 3.10). It is an industrial tool capable of cutting hard and soft materials. It utilises a high-pressure jet of water or a mixture of water and an abrasive to ensure no delamination of the composite material.

Figure 3.10: Water jet cutting machine for cutting of the holes (Flow Waterjets, USA)

Each sample was then polished on one side using a super medium (blue) and superfine (grey) diamond rubber wheel (Ernst Vetter GmbH EVE, Germany) at 5000 rpm for 25 seconds (Figure 3.11).

Figure 3.11: Super medium (blue) and fine (grey) diamond rubber wheel (EVE, Germany)

Each sample was suspended to the inner surface of a sterile bottle cap using dental floss that passed through the created to hold the sample (Figure 3.12).

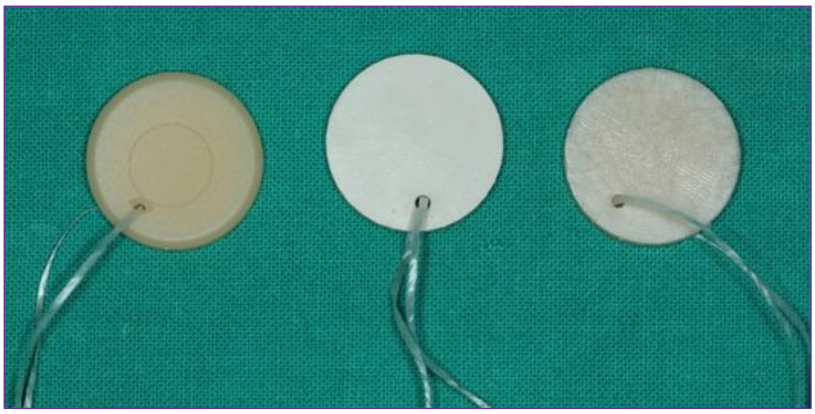


Figure 3.12: Samples with dental floss passing through the holes

A sticker was placed on the dental floss facing the unpolished surface to differentiate between the polished and unpolished surfaces (Figure 3.13). The bottles were then divided into three groups based on the material and each bottle was labelled with a different number from (1-60) and recorded on a sheet (Figure 3.14).

Figure 3.13: Sticker placed on the dental floss facing the unpolished surface

Figure 3.14: Bottles divided into three groups based on the material and labelled accordingly

The bottles were then re-sterilized in an autoclave (Hirayama Manufacturing Corporation) with the samples inside the bottles at 121°C. The samples were used in the experiment without pre-soaking or pre-coating with saliva as this would interfere with the properties of the materials, contributing to the adhesion of *Streptococcus mutans* (*S. mutans)* as suggested by Lassila *et al.* (2009).

3.3.2. Surface Characterization Using Scanning Electron Microscopy

To determine the surface roughness of the materials, each material was labelled with the name of the material. Quorum Sputter Coater (Q 150T ES)(Figure 3.15) was used for coating the samples with gold palladium to prepare the samples to be analyzed with scanning electron microscopy (SEM).

Figure 3.15: Quorum Sputter Coater (Q 150T ES)

Aluminium stubs were used to mount the samples. Carbon tabs were placed on the stubs in order for the sample to stick to the stub. The samples were then coated with gold palladium for 60 seconds (Figure 3.16).

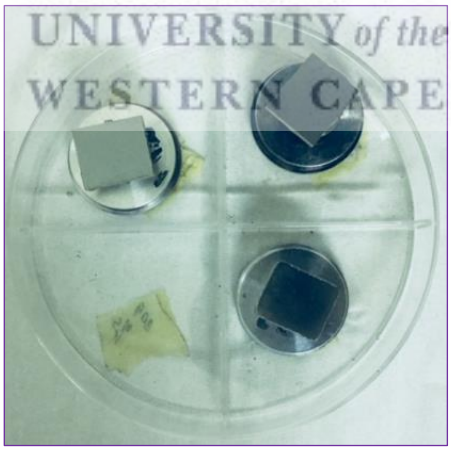


Figure 3.16: Samples coated with gold palladium

Scanning electron microscopy (SEM) (Figure 3.17) was used for the analysis of both polished and unpolished surfaces of samples. SEM images were obtained in a Field Emission SEM (SmartSEM, Zeiss, Germany) operating at 5 kV and 10 μA. Magnifications ranged from 100X to 3000X.

Figure 3.17: SEM and EDS used for the analysis of the samples

Material topography was evaluated through qualitative assessment of the surface characteristics of different materials observed via SEM imaging (Kim *et al.*, 2017). Furthermore, each sample was analyzed using Energy Dispersive X-ray Spectroscopy (EDS) using AZTEC software. EDS is an analytical technique used for the elemental analysis or chemical characterization of a sample.

3.3.3. Baseline Readings of the Samples

The colour of each sample was recorded as a baseline reading using a spectrophotometer (CM-2600d Konica, Minolta, Japan) (Figure 3.18).

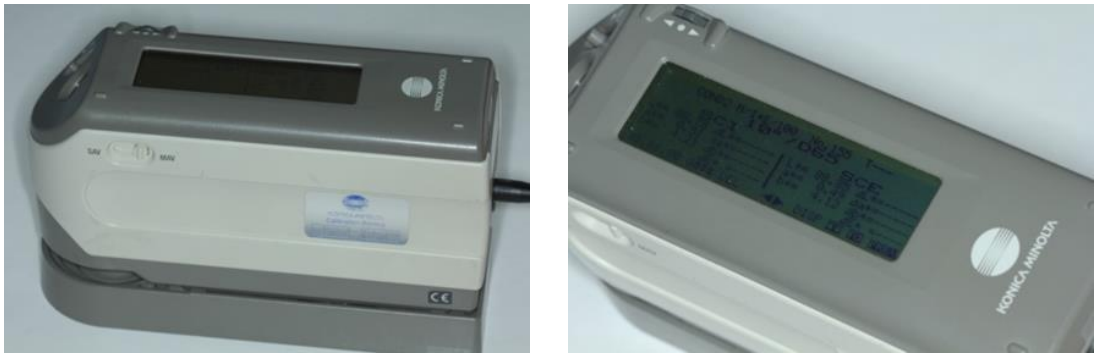


Figure 3.18: Spectrophotometer (Konica Minolta, Japan) used to measure the colour intensity of the samples

The spectrophotometer was turned on and left to warm for up to 10 minutes. The condition mode (condition 2) was selected and the instrument was set on zero calibration to allow the instrument to adapt to the ambient temperature where the measurement was performed. After the instrument was sufficiently acclimatized to the ambient temperature, it was placed on the correct white calibration plate supplied with the instrument and white calibration was performed.

The samples were placed on a flat surface with a white background. Three readings for the L*, a* and b* axis was recorded for each sample from the polished and unpolished surfaces as a baseline reading.

3.3.4. Disclosing Agent

For identification of biofilm formation on the samples, a disclosing agent (Trace disclosing tablets, young, USA) was used. The disclosing solution was prepared by dissolving 600 mg of Trace disclosing tabs in 100 ml of deionized water (Figure 3.19).

Figure 3.19: Disclosing solution (Trace Young, USA) used for staining of the biofilm

For the control group, the baseline samples were exposed to stain (disclosing solution) without bacteria to exclude any effect the staining may cause on changing the colour. The samples were placed on a flat surface with a white background. Three readings for the L*, a* and b* axis were recorded for each sample from the polished and unpolished surfaces using a spectrophotometer and was compared with the baseline readings before exposure to the stain.

Following staining, the two pulsating devices were used to clean the samples and three readings for the L*, a* and b* axis were recorded for each sample from the polished and unpolished surfaces using a spectrophotometer and compared with the baseline readings before exposure to the disclosing agent to make sure that the disclosing agent did not change the colour of the sample.

Then, the samples on the test tubes were re-sterilized using the ethylene oxide gas with the samples inside the tube.

3.3.5. Biofilm Formation

Streptococcus mutans (ATCC 25175) (American type culture collection, Rockville, MD) was incubated in brain heart infusion broth (BHI) at 37°C for 24 hours. Following the overnight culture, the bacteria were subcultured in brain heart infusion agar plates at 37°C for 24 hours. After a 24-hour incubation period the microbial cells were suspended in phosphate buffer saline solution and the concentration was adjusted to 0.5 McFarland standard (Mcf) using DensiCHEK Plus BioMérieux, Inc., Durham, USA.

A volume of 25 ml of sterile brain heart infusion broth was decanted in each bottle to which 250 µl of the 0.5 Mcf was added. The bottles were then incubated for 6 days at 37°C in an incubator. After a 48-hour incubation period, the BHI was replaced by fresh and sterile BHI media and re-incubated to allow biofilm formation.

3.3.6. Assessment of Biofilm Formation

Following the 6 days incubation the ability of *S. mutans* to form biofilm on the surface of the tested materials was evaluated by immersing all samples in the same disclosing solution. The samples were immersed for one minute to stain the *S. mutans* biofilm. The samples were allowed to air-dry under the laminar flow (Bio-Flow, Model No. 660, South Africa) for 4 hours (Figure 3.20). To evaluate the density of the biofilm formation in the three materials, the colour of the stain in each sample was measured using a spectrophotometer by placing the samples on a flat surface with white background. Three readings for the L*, a* and b* axis were recorded for each sample from the polished and unpolished surfaces.

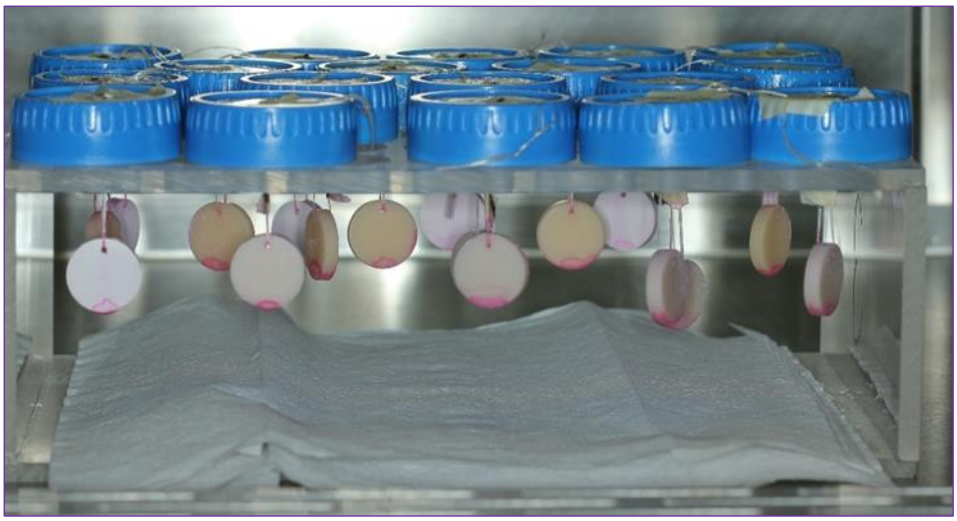


Figure 3.20: Samples allowed to air-dry under the laminar flow (Bio-Flow)

3.3.7. Evaluation of the Cleaning Efficacy of Oral Irrigating Devices

Each group was divided randomly into two subgroups (n=10). One subgroup was subjected to Aquaflosser and the other subgroup was subjected to Waterpik to clean the samples. Each sample was held by a tweezer and placed in a stand for cleaning. Each cleaning device was filled with deionized water and used according to the manufacturer's instructions.

Each device was set on a medium pressure setting (70 psi). A standard jet tip was used in each device and the tip was placed at a 45° angle and 1 cm away from the sample to simulate the action if used intraorally. Each sample was cleaned for 1 minute each for both polished and unpolished surfaces. To evaluate the efficacy of each device, the density of the biofilm following cleaning was measured using a spectrophotometer by placing the samples on a flat surface with a white background. Three readings for the L*, a* and b* axis were recorded for each sample from the polished and unpolished surfaces.

3.4. Data Analysis

Data was collected at Tygerberg Hospital using spectrophotometer and the data was transferred to Excel spreadsheet. The data was then analyzed using SPSS version 25 (IBM, USA). The mean values for each referral were then compared on the L*, a* and b* scale. A One-Way Analysis of Variance (ANOVA) was carried out to investigate if statistically significant differences occurred between all three materials (ZrO_2, LDS & PEEK) at a significance level of $p \leq 0.05$. Once it was assumed that the data met the assumption of homogeneity of variances, a single-step multiple comparison procedure and statistical test Tukey's post hoc test (HSD) was then performed to compare all possible pairs of means that existed between all three materials at a significance level of $p \leq 0.05$ (ANOVA is a broad analysis, while Tukey's test is more specific).

The ANOVA and Tukey's tests were used to compare the mean values of ΔE* as well as the mean values of L*, a* and b* for all three materials before culturing the *S. mutans* (at baseline), after culturing in a media containing *S. mutans* (after biofilm formation) and after cleaning with the two pulsating devices (Aquaflosser (AQ) and Waterpik (WP)). To calculate the overall colour difference (ΔE*) the L*, a* and b* values were then used in the following equation:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Where ΔE*stand for the colour difference and ΔL*, Δ a*, Δb*, L lightness, a red-green coordinate, and b yellow-blue coordinate, respectively.

CHAPTER 4

RESULTS

4.1 Evaluation of the Disclosing Agent on All Three Materials

To determine if the disclosing agent had any effect on the materials (control) before culturing the *S. mutans* (at baseline), the mean values of ΔE* were compared for each of the three materials at baseline with the materials after immersion the disclosing agent, and after cleaning with the two pulsating devices (AQ and WP). There were no colour differences between the materials at baseline (BL) and following cleaning with the two pulsating devices AQ and WP.

4.2 Evaluation of Biofilm Formation on the Surfaces of PEEK, ZrO_2 and LDS

The ability of *S. mutans* to form biofilm on the surfaces of PEEK, ZrO_2 and LDS was evaluated by measuring the mean values of ΔE* before (baseline) and after culturing in media containing *S. mutans* (biofilm formation) for all three materials. Fifty-four samples were used in this test, each group contained eighteen samples for each material (n=18). The colour of each sample was recorded before and after biofilm formation. This was done for each polished and unpolished surface.

4.2.1 Polished Surfaces

There were statistically significant differences (ANOVA $p \leq 0.05$) in the colour readings for all three materials when comparing the three materials at baseline with the materials after biofilm formation. PEEK showed the highest colour change with a mean value of

12.63 compared to LDS with a mean value of 6.99 and ZrO_2 with the lowest mean value colour change of 6.31 (Figure 4.1).

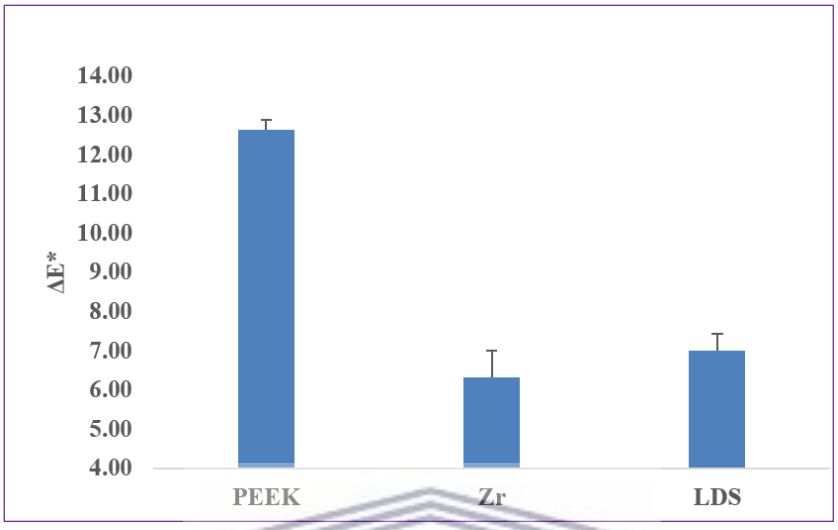


Figure 4.1: Comparison of the mean values of ΔE* between all three materials at baseline and after biofilm formation

There were statistically significant differences between PEEK and LDS (Tukey test, $p \leq 0.05$) and PEEK and ZrO_2 (Tukey test, $p \leq 0.05$). There was no statistically significant difference between ZrO_2 and LDS (Tukey test, $p > 0.05$) (Table 4.1).

Table 4.1: Comparison of the mean values of ΔE* between each pair of the three materials at baseline and after biofilm formation

Dependent Variable			Mean Difference (I-J)	Std. Error	95% CI		P value
					Lower	Upper	
ΔE* Baseline vs Biofilm formation	PEEK	ZrO_2	6.32	0.68	4.65	7.98	0.000
		LDS	5.63	0.68	3.97	7.30	0.000
	ZrO_2	PEEK	-6.32	0.68	-7.98	-4.65	0.000
		LDS	-0.68	0.68	-2.34	0.98	0.585
	LDS	PEEK	-5.63	0.68	-7.30	-3.97	0.000
		ZrO_2	0.68	0.68	-0.98	2.34	0.585

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$; CI: Confidence interval

4.2.2 Unpolished Surfaces

There were statistically significant differences (ANOVA $p \leq 0.05$) in the colour readings for all three materials when comparing the three materials at baseline with the materials after biofilm formation, where PEEK showed the highest colour change with a mean value of 12.64, followed by ZrO2 with a mean value of 7.66 and LDS with the lowest mean value colour change of 6.60 (Figure 4.2).

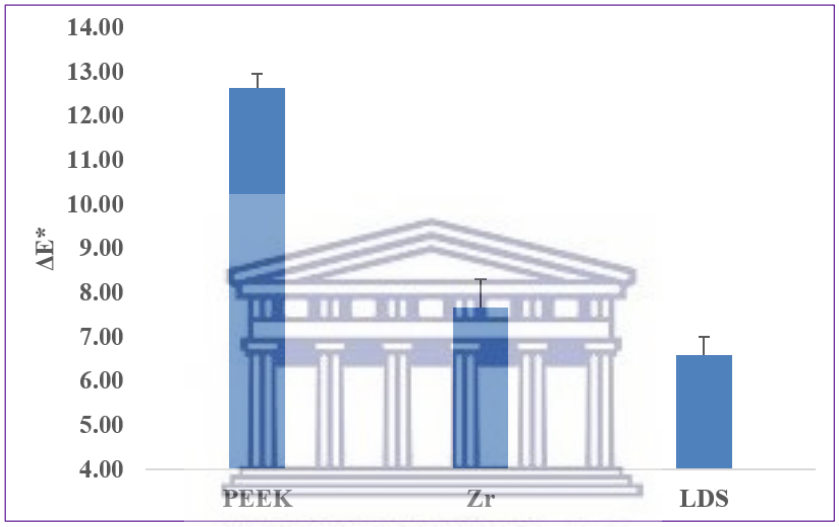


Figure 4.2: Comparison of the mean values of ΔE* between all three materials at baseline and after biofilm formation

There were statistically significant differences between PEEK and LDS (Tukey test, $p \leq 0.05$) and PEEK and ZrO_2 (Tukey test, $p \leq 0.05$). There was no statistically significant difference between ZrO_2 and LDS (Tukey test, $p > 0.05$) (Table 4.2).

Table 4.2: Comparison of the mean values of ΔE* between each pair of the three materials at baseline and after biofilm formation

Dependent Variable			Mean Difference (I-J)	Std. Error	95% CI		P value
					Lower	Upper	
ΔE* Baseline vs Biofilm formation	PEEK	ZrO_2	4.97	0.66	3.36	6.59	0.000
		LDS	6.03	0.66	4.42	7.65	0.000
	ZrO_2	PEEK	-4.97	0.66	-6.59	-3.36	0.000
		LDS	1.06	0.66	-0.55	2.67	0.261
	LDS	PEEK	-6.04	0.66	-7.65	-4.42	0.000
		ZrO_2	-1.06	0.66	-2.67	0.55	0.261

Differences statistically significant at p≤0.05; Statistically not significant p>0.05; CI: Confidence interval

4.3 Evaluation of All Three Materials at Baseline, After Biofilm Formation and After Cleaning with Aquaflosser and Waterpik

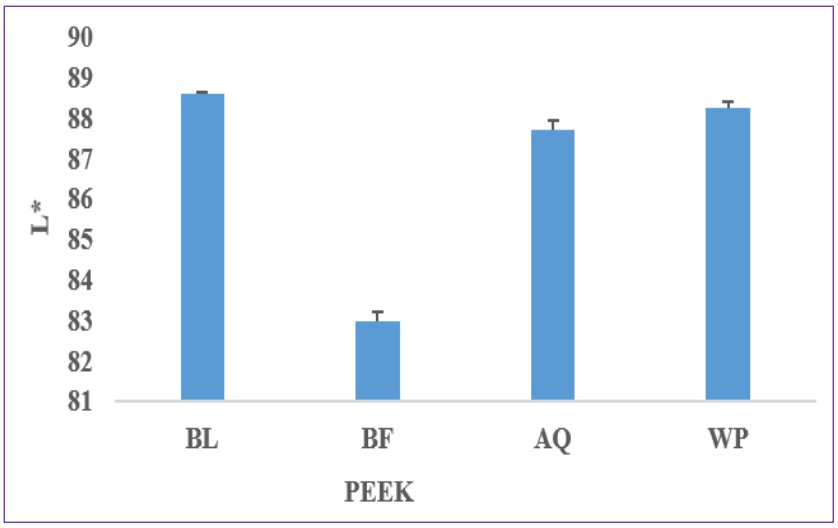
There were colour differences in ΔE* between all three materials at baseline and after biofilm formation. In order to determine exactly where the colour difference exists, the mean values of L*, a* and b*for each of the three materials at baseline, after biofilm formation and after cleaning with AQ and WP were measured and compared on L*, a*, b* scale to give an indication in which direction the colour difference exists.

4.3.1 Polished Surfaces

4.3.1.1 Polyetheretherketone (PEEK)

4.3.1.1.1 Colour Differences Between The Mean Values in L* Scale

There was statistically significant difference (ANOVA p≤0.05) in the L* coordinate for PEEK at baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.3).



BL=Baseline, BF=Biofilm formation, AQ=Aquaflosser and WP=Waterpik

Figure 4.3: Comparison between the mean values in L* scale for PEEK at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were statistically significant differences (Tukey test, p≤0.05) between the material at baseline 88.61 with the material after biofilm formation 82.98 and after cleaning with AQ 87.72. No statistically significant differences (Tukey test, p>0.05) were found between the material at baseline 88.61 and after cleaning with WP 88.26 (Table 4.3).

Table 4.3: Comparison between the mean values in L* scale for PEEK at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
L*	Baseline	Biofilm formation	5.62	0.21	5.04	6.20	0.00
		Aquaflosser (AQ)	0.88	0.26	0.17	1.60	0.00
		Waterpik (WP)	0.34	0.26	-0.36	1.06	0.573

Differences statistically significant at p≤0.05; Statistically not significant p>0.05

4.3.1.1.2 Colour Differences Between the Mean Values in a* Scale

There was statistically significant difference (ANOVA p≤0.05) in the a* coordinate for PEEK at baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.4).

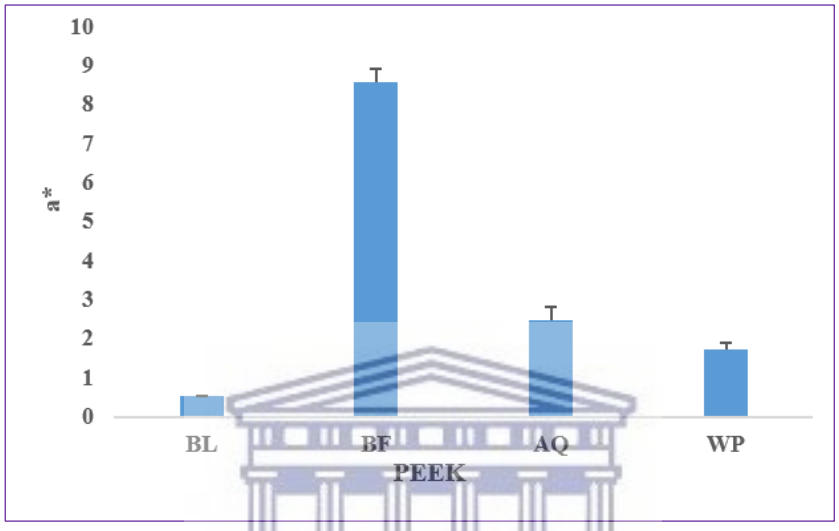


Figure 4.4: Comparison between the mean values in a* scale for PEEK at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were statistically significant differences (Tukey test, p≤0.05) between the material at baseline 0.52 with the material after biofilm formation 8.58 and after cleaning with AQ 2.49and WP 1.74 (Table 4.4).

Table 4.4: Comparison between the mean values in a* scale for PEEK at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
a*	Baseline	Biofilm formation	-8.06	0.30	-8.88	-7.25	0.00
		Aquaflosser (AQ)	-1.97	0.37	-2.97	-0.96	0.00
		Waterpik (WP)	-1.22	0.37	-2.22	-0.21	0.012

Differences statistically significant at p≤0.05; Statistically not significant p>0.05

4.3.1.1.3 Colour Differences Between the Mean Values in b*Scale

There was statistically significant difference (ANOVA p≤0.05) in the b* coordinate for PEEK at baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.5).

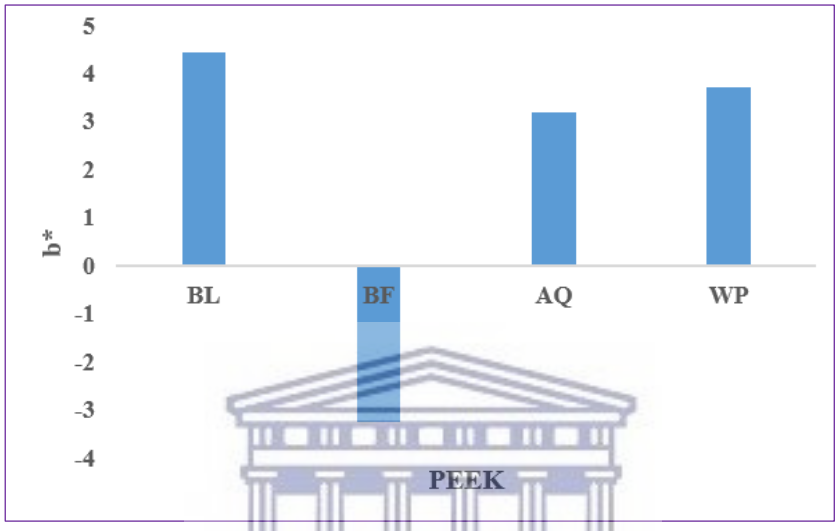


Figure 4.5: Comparison between the mean values in b* scale for PEEK at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, p≤0.05) between the mean of the material at baseline 4.46 with the material after biofilm formation -3.25 and after cleaning with AQ 3.19 and WP 3.71 (Table 4.5).

Table 4.5: Comparison between the mean values in b* scale for PEEK at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
b*	Baseline	Biofilm formation	7.72	0.28	7.11	8.32	0.00
		Aquaflosser (AQ)	1.26	0.28	0.51	2.01	0.00
		Waterpik (WP)	0.74	0.28	-0.00	1.49	0.00

Differences statistically significant at p≤0.05; Statistically not significant p>0.05

4.3.1.2 Zirconium dioxide (ZrO_2)

4.3.1.2.1 Colour Differences Between the Mean Values in L* Scale

There was statistically significant difference (ANOVA $p \leq 0.05$) in the L* coordinate for ZrO_2 at baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.6).

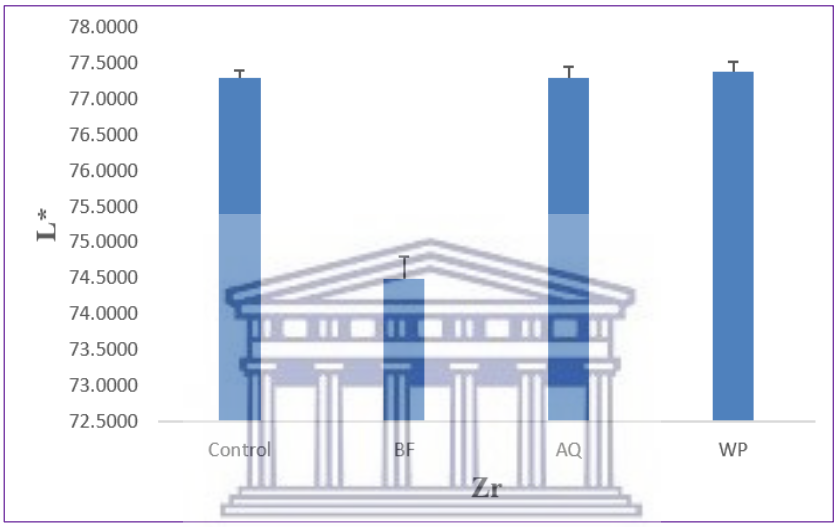


Figure 4.6: Comparison between the mean values in L* scale for ZrO_2 at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, $p \leq 0.05$) between the mean of the material at baseline 77.29 with the material after biofilm formation 74.48. However, no statistically significant differences (Tukey test, $p > 0.05$) were found between the material at baseline 77.29 and after cleaning with AQ 77.29 and WP 77.37 (Table 4.6).

Table 4.6: Comparison between the mean values in L* scale for ZrO_2 at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
L*	Baseline	Biofilm formation	2.81	0.27	2.07	3.55	0.00
		Aquaflosser (AQ)	-0.00	0.34	-0.90	0.91	1.00
		Waterpik (WP)	-0.07	0.34	-0.98	0.83	0.996

Differences statistically significant at p≤0.05; Statistically not significant p>0.05

4.3.1.2.2 Colour Differences Between the Mean Values in a* Scale

There was statistically significant difference (ANOVA p≤0.05) in the a* coordinate for ZrO_2 at the baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.7).

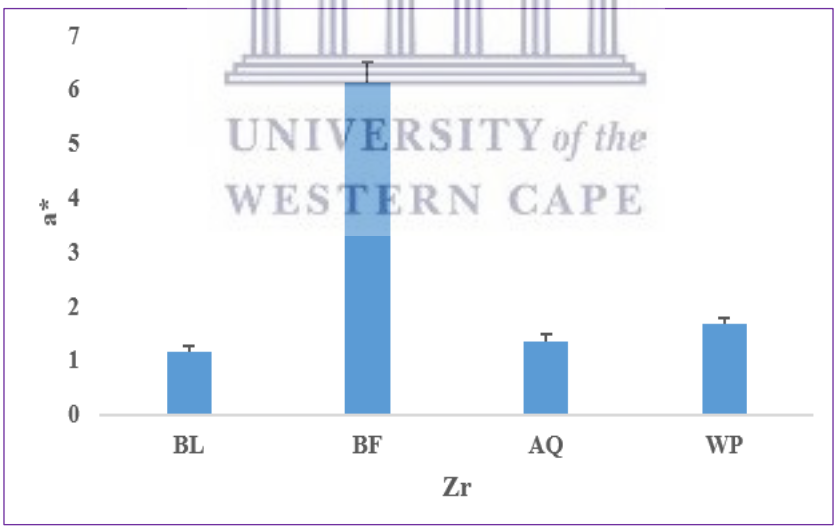


Figure 4.7: Comparison between the mean values in a* scale for ZrO_2 at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, $p \leq 0.05$) between the mean of the material at baseline 1.17 with the material after biofilm formation 6.13. However, no statistically significant differences (Tukey test, $p > 0.05$) were found between the material at baseline 1.17 and after cleaning with AQ 1.36 and WP 1.67 (Table 4.7).

Table 4.7: Comparison between the mean values in a* scale for ZrO_2 at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
a*	Baseline	Biofilm formation	-4.95	0.31	-5.78	-4.13	0.00
		Aquaflosser (AQ)	-0.188	0.38	-1.21	0.83	0.961
		Waterpik (WP)	-0.50	0.38	-1.52	0.52	0.564

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$

4.3.1.2.3 Colour Differences Between the Mean Values in b* Scale

There was statistically significant difference (ANOVA $p \leq 0.05$) in the b* coordinate for ZrO_2 at the baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.8).

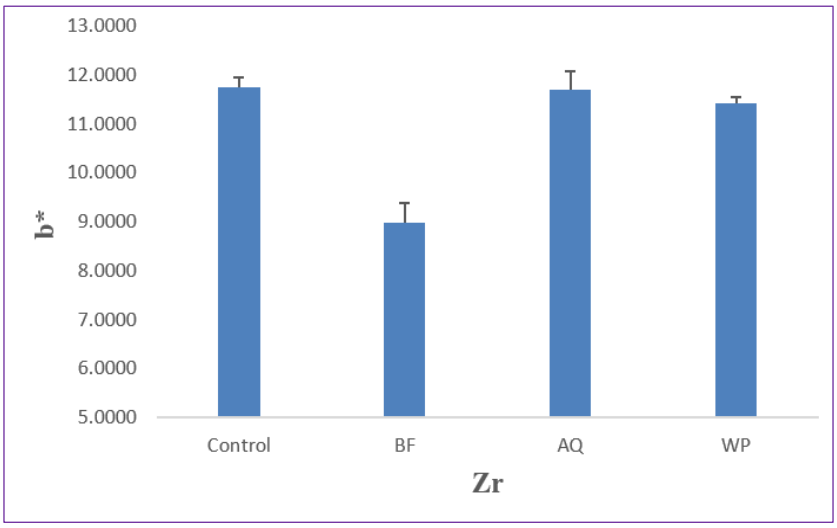


Figure 4.8: Comparison between the mean values in b* scale for ZrO_2 at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, $p \leq 0.05$) between the mean of the material at baseline 11.73 with the material after biofilm formation 8.96. However, no statistically significant differences (Tukey test, $p > 0.05$) between the material at baseline 11.73 and after cleaning with AQ 11.68 and WP 11.40 (Table 4.8).

Table 4.8: Comparison between the mean values in a* scale for ZrO_2 at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
b*	Baseline	Biofilm formation	-2.76	0.41	1.65	3.87	0.00
		Aquaflosser (AQ)	0.05	0.51	-1.31	1.41	1.000
		Waterpik (WP)	0.32	0.51	-1.04	1.69	0.920

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$

4.3.1.3 Lithium Disilicate (LDS)

4.3.1.3.1 Colour Differences Between the Mean Values in L* Scale

There was statistically significant difference (ANOVA $p \leq 0.05$) in L* coordinate for LDS at the baseline with the material after biofilm formation and after application of the two pulsating devices AQ and WP (Figure 4.9).

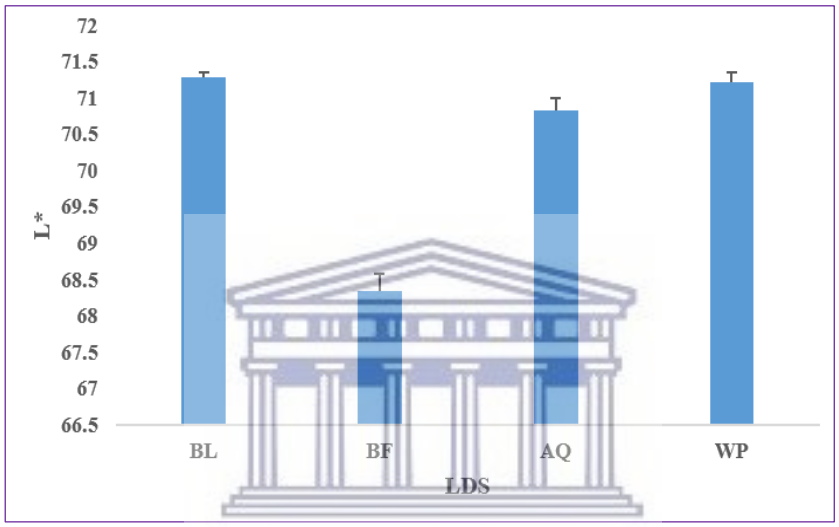


Figure 4.9: Comparison between the mean values in L* scale for LDS at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, $p \leq 0.05$) between the mean of the material at baseline 71.29 with the material after biofilm formation 68.34. However, no statistically significant differences (Tukey test, $p > 0.05$) were found between the material at baseline 71.29 and after cleaning with AQ 70.84 and WP 71.23 (Table 4.9).

Table 4.9: Comparison between the mean values in L* scale for LDS at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
L*	Baseline	Biofilm formation	2.95	0.22	2.36	3.54	0.00
		Aquaflosser (AQ)	0.45	0.27	-0.27	1.17	0.359
		Waterpik (WP)	0.05	0.27	-0.66	08	0.997

Differences statistically significant at p≤0.05; Statistically not significant p>0.05

4.3.1.3.2 Colour Differences Between the Mean Values in a* Scale

There was statistically significant difference (ANOVA p≤0.05) in the a* coordinate for LDS at the baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.10).

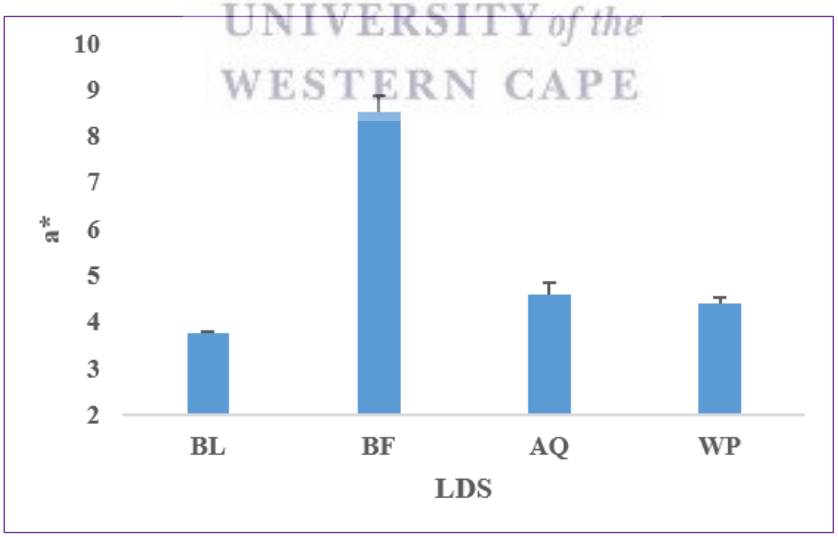


Figure 4.10: Comparison between the mean values in a* scale for LDS at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, p≤0.05) between the mean of the material at baseline 3.78 with the material after biofilm formation 8.54. However, no statistically significant differences (Tukey test, p>0.05) were found between the material at baseline 3.93 and after cleaning with AQ 4.61 and WP 4.40 (Table 4.10).

Table 4.10: Comparison between the mean values in a* scale for LDS at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
a*	Baseline	Biofilm formation	-4.76	0.28	-5.53	-3.99	0.00
		Aquaflosser (AQ)	-0.83	0.35	-1.78	0.11	0.105
		Waterpik (WP)	-0.62	0.35	-1.57	0.32	0.314

Differences statistically significant at p≤0.05; Statistically not significant p>0.05

4.3.1.3.3 Colour Differences Between the Mean Values in b*Scale

There was statistically significant difference (ANOVA p≤0.05) in the **b*** coordinate for LDS at the baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.11).

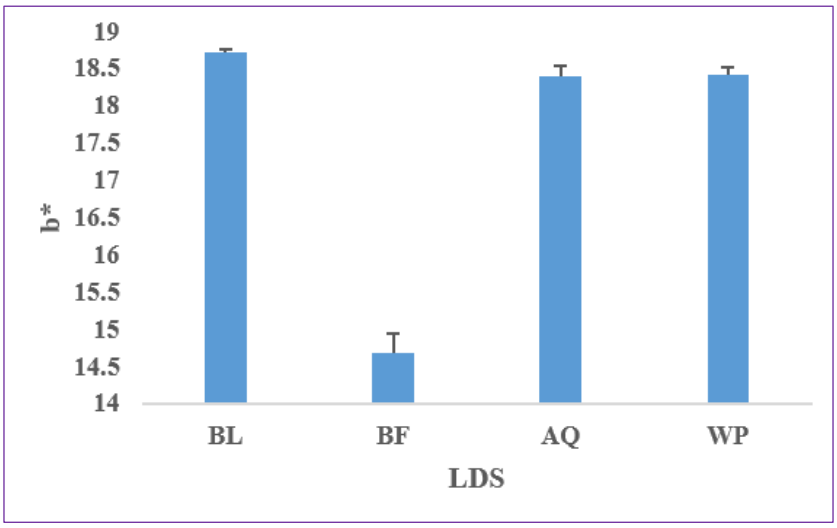


Figure 4.11: Comparison between the mean values of b* for LDS at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, p≤0.05) between the mean of the material at baseline 18.71 with the material after biofilm formation 14.68. However, no statistically significant differences (Tukey test, p>0.05) were found between the material at baseline 18.71 and after cleaning with AQ 18.39 and WP 18.42 (Table 4.11).

Table 4.11: Comparison between the mean values in b*scale for LDS at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
b*	Baseline	Biofilm formation	4.02	0.22	3.43	4.62	0.00
		Aquaflosser (AQ)	0.32	0.27	-0.41	1.06	0.661
		Waterpik (WP)	0.29	0.27	-0.44	1.03	0.724

Differences statistically significant at p≤0.05; Statistically not significant p>0.05

4.3.2 Unpolished Surfaces

4.3.2.1 Polyetheretherketone (PEEK)

4.3.2.1.1 Colour Differences Between the Mean Values in L* Scale

There was statistically significant difference (ANOVA $p \leq 0.05$) in the L* coordinate for PEEK at the baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.12).

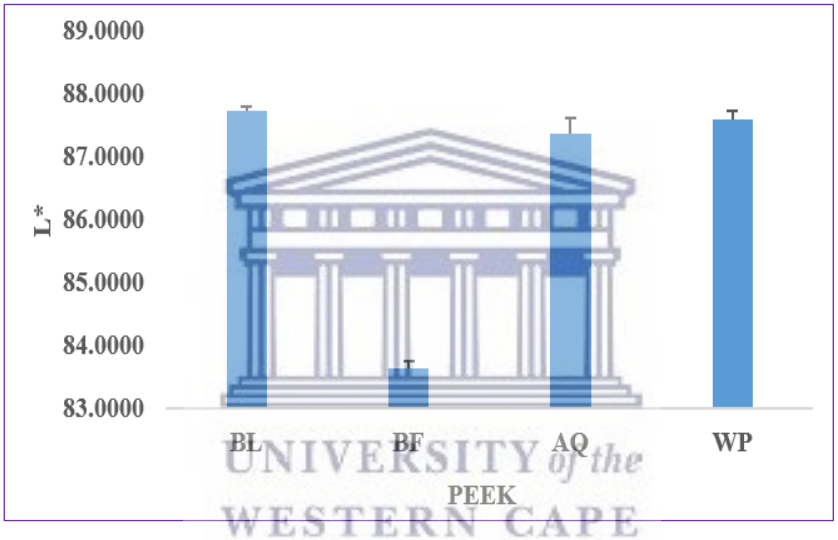


Figure 4.12: Comparison between the mean values in L* scale for PEEK at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, $p \leq 0.05$) between the mean of the material at baseline 87.71 with the material after biofilm formation 83.63. However, no statistically significant differences (Tukey test, $p > 0.05$) were found between the material at baseline 87.71 and after cleaning with AQ 87.36 and WP 87.59 (Table 4.12).

Table 4.12: Comparison between the mean values in L* scale for PEEK at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
L*	Baseline	Biofilm formation	4.08	0.16	3.66	4.49	0.00
		Aquaflosser (AQ)	0.35	0.19	-0.16	0.86	0.277
		Waterpik (WP)	0.12	0.19	-0.39	0.63	0.924

Differences statistically significant at p≤0.05; Statistically not significant p>0.05

4.3.2.1.2 Colour Differences Between the Mean Values in a* Scale

There was statistically significant difference (ANOVA p≤0.05) in the a* coordinate for PEEK at baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.13).

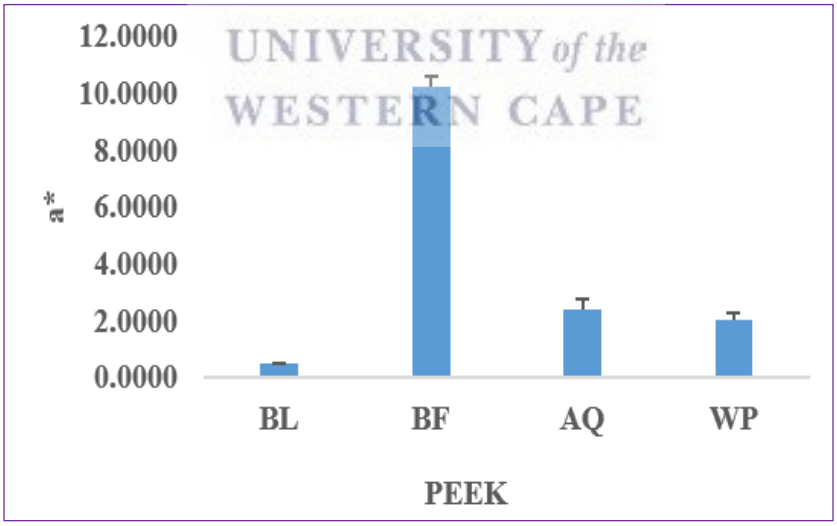


Figure 4.13: Comparison between the mean values in a* scale for PEEK at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, p≤0.05) between the mean of the

material at baseline 0.50 with the material after biofilm formation 10.22 and after cleaning with AQ 2.38 and WP 2.02 (Table 4.13).

Table 4.13: Comparison between the mean values in a* scale for PEEK at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
a*	Baseline	Biofilm formation	-9.72	0.34	-10.63	-8.80	0.00
		Aquaflosser (AQ)	-1.87	0.42	-3.00	-0.74	0.000
		Waterpik (WP)	-1.51	0.42	-2.64	-0.38	0.004

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$

4.3.2.1.3 Colour Differences Between the Mean Values in b* Scale

There was statistically significant difference (ANOVA p≤0.05) in the b* coordinate for PEEK at baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.14).

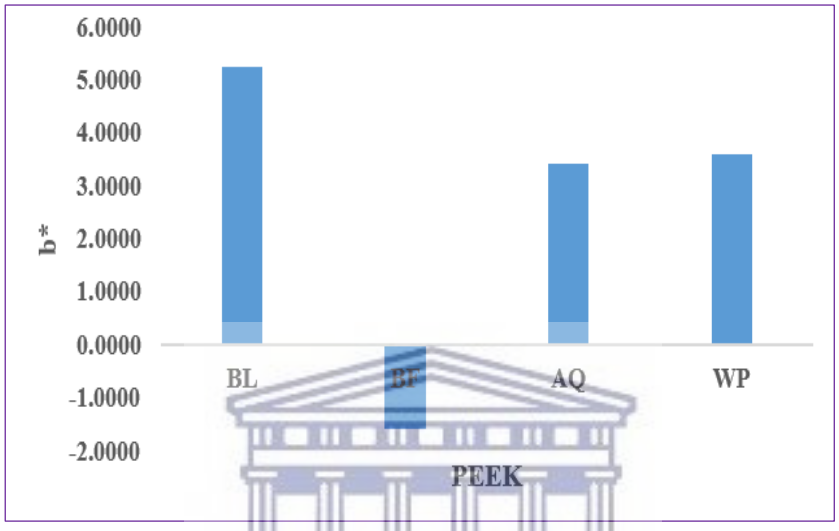


Figure 4.14: Comparison between the mean values in b* scale for PEEK at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, p≤0.05) between the mean of the material at baseline 5.26 with the material after biofilm formation -1.58 and after cleaning with AQ 3.41 and WP 3.60 (Table 4.14).

Table 4.14: Comparison between the mean values in b* scale for PEEK at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
b*	Baseline	Biofilm formation	6.84	0.21	6.28	7.40	0.00
		Aquaflosser (AQ)	1.84	0.26	1.14	2.53	0.000
		Waterpik (WP)	1.65	0.26	0.96	2.35	0.000

Differences statistically significant at p≤0.05; Statistically not significant p>0.05

4.3.2.2 Zirconium Dioxide (ZrO2)

4.3.2.2.1 Colour Differences Between the Mean Values in L* Scale

There was statistically significant difference (ANOVA $p \leq 0.05$) in the L* coordinate for ZrO_2 at baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.15).

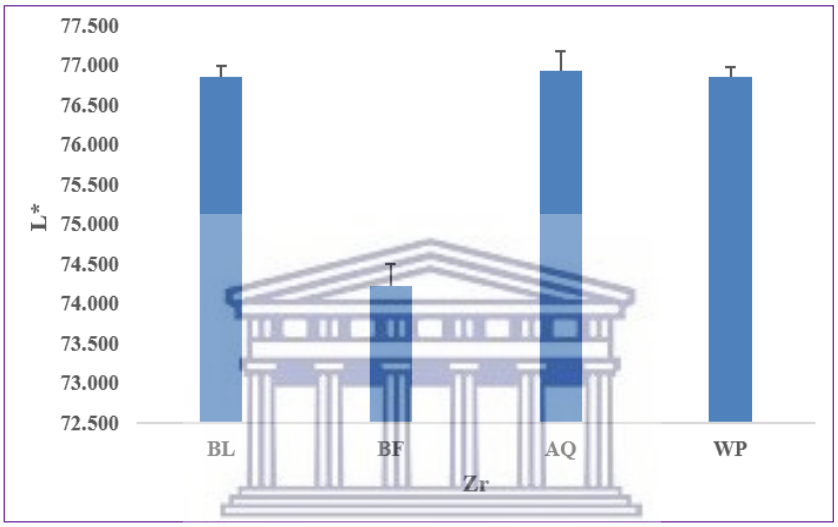


Figure 4.15: Comparison between the mean values in L* scale for ZrO_2 at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, $p \leq 0.05$) between the mean of the material at baseline 76.85 with the material after biofilm formation 74.22. However, no statistically significant differences (Tukey test, $p > 0.05$) were found between the material at baseline 76.85 and after cleaning with AQ 76.93 and WP 76.85 (Table 4.15).

Table 4.15: Comparison between the mean values in L* scale for ZrO_2 at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
L*	Baseline	Biofilm formation	2.62	0.27	1.90	3.35	0.00
		Aquaflosser (AQ)	-0.08	0.33	-0.98	0.81	0.994
		Waterpik (WP)	0.00	0.33	-0.89	0.89	1.000

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$

4.3.2.2.2 Colour Differences Between the Mean Value in a* Scale

There was statistically significant difference (ANOVA $p \leq 0.05$) in the a* coordinate for ZrO_2 at the baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.16).

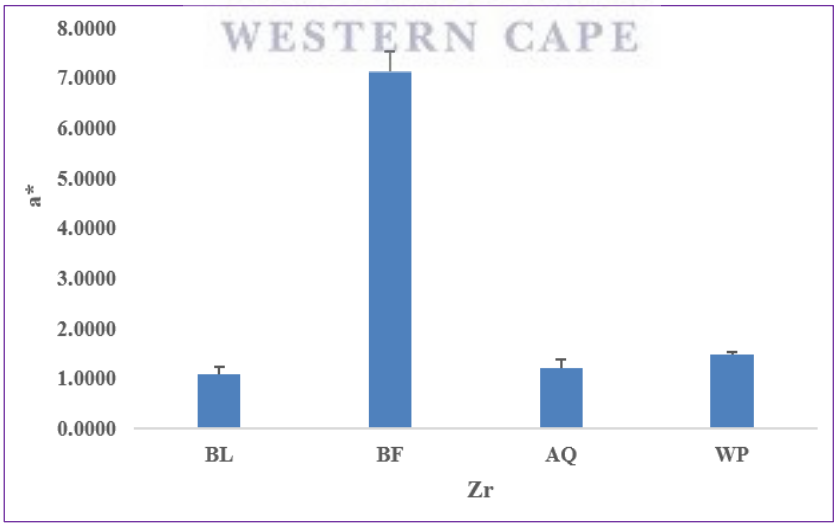


Figure 4.16: Comparison between the mean values in a* scale for ZrO_2 at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, p≤0.05) between the mean of the material at baseline 1.10 with the material after biofilm formation 7.13. However, no statistically significant differences (Tukey test, p>0.05) were found between the material at baseline 1.10 and after cleaning with AQ 1.20 and WP 1.48 (Table 4.16).

Table 4.16: Comparison between the mean values in a* scale for ZrO_2 at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
a*	Baseline	Biofilm formation	-6.03	0.34	-6.94	-5.11	0.00
		Aquaflosser (AQ)	-0.10	0.42	-1.23	1.02	0.995
		Waterpik (WP)	-0.38	0.42	-1.51	0.75	0.808

Differences statistically significant at p≤0.05; Statistically not significant p>0.05

4.3.2.2.3 Colour Differences Between the Mean Values in b* Scale

There was statistically significant difference (ANOVA p≤0.05) in the b*coordinate for ZrO_2 at baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.17).

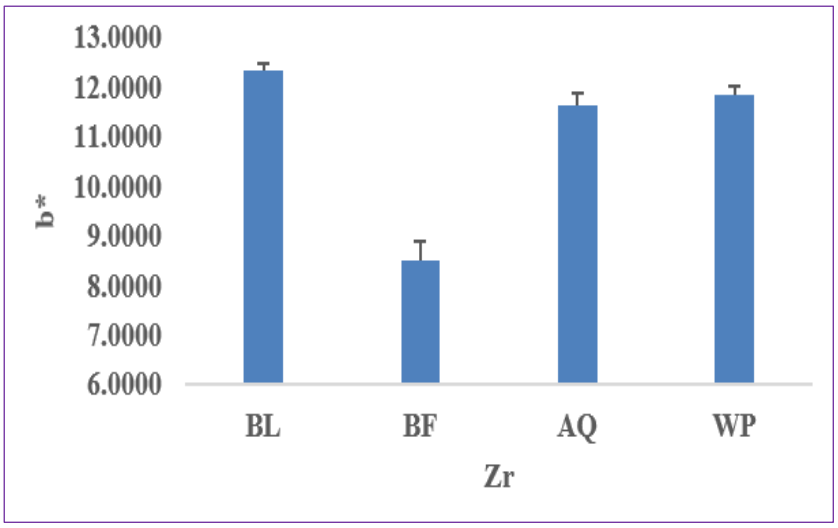


Figure 4.17: Comparison between the mean values in b* scale for ZrO_2 at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, $p \leq 0.05$) between the mean of the material at baseline 12.33 with the material after biofilm formation 8.49. However, no statistically significant differences (Tukey test, $p > 0.05$) were found between the material at baseline 12.33 and after cleaning with AQ 11.62 and WP 11.84 (Table 4.17).

Table 4.17: Comparison between the mean values in b* scale for ZrO_2 at baseline with the material after biofilm formation and after cleaning with AQ & WP.

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
b*	Baseline	Biofilm formation	3.83	0.36	2.87	4.80	0.00
		Aquaflosser (AQ)	0.70	0.45	-0.48	1.89	0.403
		Waterpik (WP)	0.49	0.45	-0.70	1.68	0.695

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$

4.3.2.3 Lithium Disilicate (LDS)

4.3.2.3.1 Colour Differences Between the Mean Values in L* Scale

There was statistically significant difference (ANOVA $p \leq 0.05$) in the L* coordinate for LDS at baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.18).

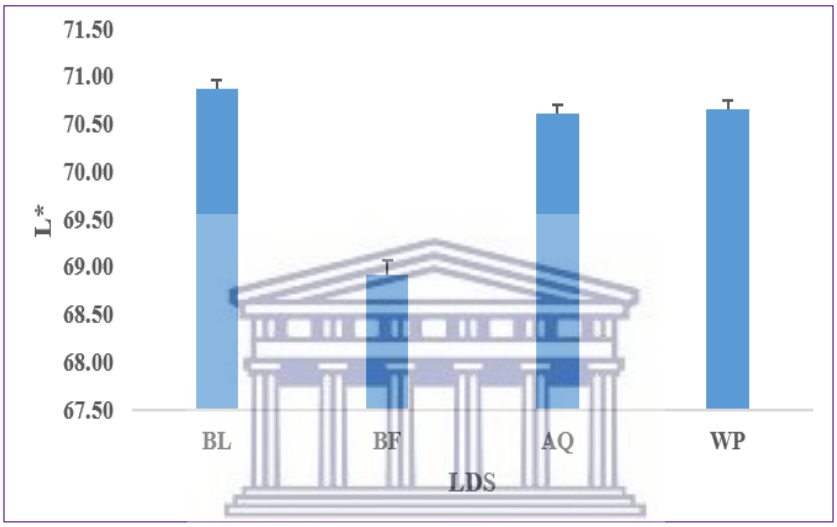


Figure 4.18: Comparison between the mean values in L* scale for LDS at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, $p \leq 0.05$) between the mean of the material at baseline 70.87 with the material after biofilm formation 68.93. However, no statistically significant differences (Tukey test, $p > 0.05$) were found between the material at baseline 70.87 and after cleaning with AQ 70.61 and WP 70.66 (Table 4.18).

Table 4.18: Comparison between the mean values in L* scale for LDS at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
L*	Baseline	Biofilm formation	1.94	0.16	1.53	2.35	0.00
		Aquaflosser (AQ)	0.26	0.19	-0.25	0.77	0.540
		Waterpik (WP)	0.21	0.19	-0.30	0.72	0.695

Differences statistically significant at p≤0.05; Statistically not significant p>0.05

4.3.2.3.2 Colour Differences Between the Mean Values in a* Scale

There was statistically significant difference (ANOVA p≤0.05) in the a* coordinate for LDS at baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.19).

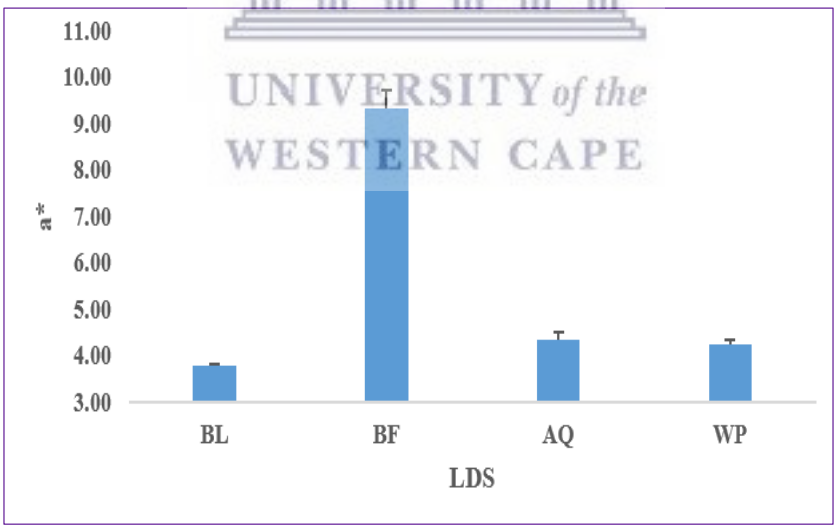


Figure 4.19: Comparison between the mean values of a* for LDS at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, p≤0.05) between the mean of the material at baseline 3.80 with the material after biofilm formation 9.35. However, no statistically significant differences (Tukey test, p>0.05) were found between the material at baseline 3.80 and after cleaning with AQ 4.36 and WP 4.26 (Table 4.19).

Table 4.19: Comparison between the mean values in a* scale for LDS at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
a*	Baseline	Biofilm formation	-5.54	0.32	-6.38	-4.70	0.00
		Aquaflosser (AQ)	-0.56	0.39	-1.59	0.47	0.482
		Waterpik (WP)	-0.46	0.39	-1.49	0.58	0.643

Differences statistically significant at p≤0.05; Statistically not significant p>0.05

4.3.2.3.3 Colour Differences Between the Mean Values of in b* Scale

There was statistically significant difference (ANOVA p≤0.05) in the b* coordinate for LDS at baseline with the material after biofilm formation and after application of the two pulsating devices (AQ and WP) (Figure 4.20).

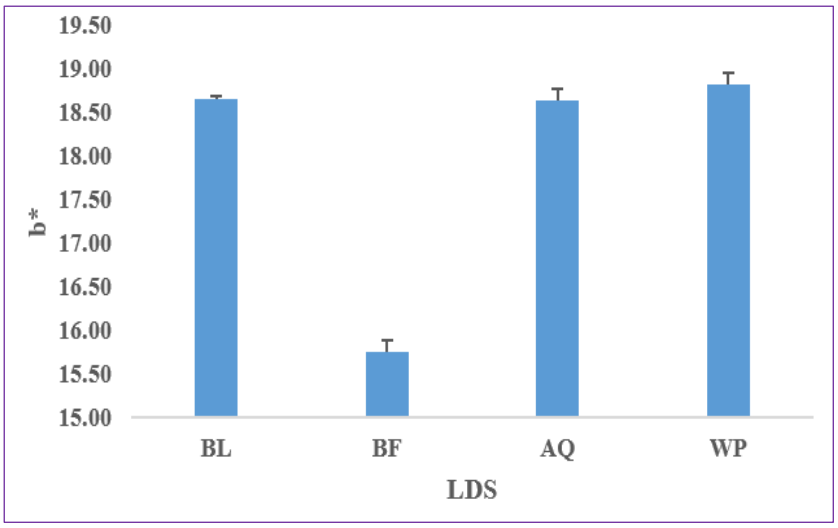


Figure 4.20: Comparison between the mean values in b* scale for LDS at baseline with the material after biofilm formation and after cleaning with AQ & WP

There were significant differences (Tukey test, $p \leq 0.05$) between the mean of the material at baseline 18.65 with the material after biofilm formation 15.75. However, no statistically significant differences (Tukey test, $p > 0.05$) were found between the material at baseline 18.65 and after cleaning with AQ 18.64 and WP 18.83 (Table 4.20).

Table 4.20: Comparison between the mean values in b* scale for LDS at baseline with the material after biofilm formation and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
b*	Baseline	Biofilm formation	2.90	0.12	2.57	3.22	0.00
		Aquaflosser (AQ)	0.01	0.15	-0.40	0.41	1.000
		Waterpik (WP)	-0.18	0.15	-0.58	0.23	0.661

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$

4.4 Evaluation of Biofilm Formation on Both Polished and Unpolished Surfaces for All Three Materials

To determine the colour intensity of stained biofilm between the polished and unpolished surfaces, the mean values of L*, a* and b* for both polished and unpolished surfaces were measured and compared for each of the three materials after culturing the materials in media containing *S. mutans* (biofilm formation).

4.4.1 Comparison of the Mean Values of L*, a* and b* Between Polished and Unpolished Surfaces of PEEK After Biofilm Formation

The mean values of L*, a* and b* were increased after biofilm formation. For L* it increased from 74.73 at baseline to 75.42 after biofilm formation, for **a*** it increased from 7.90 at baseline to 9.62 after biofilm formation, and for b* it increased from 2.44 at baseline to 4.19 after biofilm formation. There were no statistically significant differences (ANOVA $p>0.05$) between the mean values of polished and unpolished surfaces in the L* and b* coordinate. However, there was statistically significant differences ($p\leq0.05$) between the mean values of polished and unpolished surfaces in **a*** coordinate. The biofilm formation was found to be more on the unpolished surfaces than the polished surfaces (Table 4.21) for all materials.

Table 4.21: Comparison of the mean values of L*, a* and b* between polished and unpolished surfaces of PEEK after biofilm formation

Surface		Mean	Std. Error Mean	P value
L* Biofilm formation	Polished	74.73	5.64	0.932
	Unpolished	75.42	5.69	
a* Biofilm formation	Polished	7.90	0.49	0.031
	Unpolished	9.62	0.59	
b* Biofilm formation	Polished	2.44	4.02	0.756
	Unpolished	4.19	3.88	

Differences statistically significant at $p\leq0.05$; Statistically not significant $p>0.05$

4.4.2 Comparison of the Mean Values of L*, a* and b* Between Polished and Unpolished Surfaces of ZrO_2 After Biofilm Formation

The mean values of L* decreased from 67.15 at baseline to 66.87 after biofilm formation, a* increased from 5.75 at baseline to 6.78 after biofilm formation, and the b* decreased from 13.58 at baseline to 13.13 after biofilm formation. There was no statistically significant differences (ANOVA $p>0.05$) between the mean values of polished and unpolished surfaces of L*, a* and b* coordinate. However, the biofilm formation was found to be more on the unpolished surfaces than the polished (Table 4.22).

Table 4.22: Comparison between the mean values of L*, a* and b* for polished and unpolished surfaces of ZrO_2 after biofilm formation

Surface		Mean	Std. Error Mean	P value
L* Biofilm formation	Polished	67.15	5.06	0.969
	Unpolished	66.87	5.04	
a* Biofilm formation	Polished	5.75	0.40	0.097
	Unpolished	6.78	0.44	
b* Biofilm formation	Polished	13.58	3.18	0.922
	Unpolished	13.13	3.22162	

Differences statistically significant at $p\leq0.05$; Statistically not significant $p>0.05$

4.4.3 Comparison of the Mean Values of L*, a* and b* Between Polished and Unpolished Surfaces of LDS After Biofilm Formation

The mean values of L* decreased from 70.30 at baseline to 70.17, a* increased from 5.54 at baseline to 5.75 after biofilm formation, and b* increased from 17.32 at baseline to 17.75 after biofilm formation. There were no statistically significant differences (ANOVA $p>0.05$) between the mean values of polished and unpolished surfaces of L*, a* and b* coordinate (Table 4.23).

Table 4.23: Comparison between the mean values of L*, a* and b* for polished and unpolished surfaces of LDS after biofilm formation

Surface		Mean	Std. Error Mean	P value
L* Biofilm formation	Polished	70.30	0.20	0.584
	Unpolished	70.17	0.13	
a* Biofilm formation	Polished	5.54	0.30	0.663
	Unpolished	5.75	0.36	
b* Biofilm formation	Polished	17.32	0.26	0.190
	Unpolished	17.75	0.19	

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$

4.5 Assessment of the Pulsating Devices Aquaflosser and Waterpik to Remove Biofilm from the Surfaces of PEEK, ZrO2 and LDS

The ability of the pulsating devices to remove biofilm from the surfaces of PEEK, ZrO_2 and LDS was calculated by measuring the mean values of ΔE* for all three materials after biofilm formation with the materials after cleaning with AQ and WP using the formula:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

4.5.1 Polished Surfaces

The mean values of ΔE* for PEEK after biofilm formation and after cleaning with AQ and WP was measured and compared. PEEK showed the highest mean value of 10.71, followed by LDS with a mean value of 6.18 and ZrO_2 with the lowest mean value colour change of 6.05 (Figure 4.21).

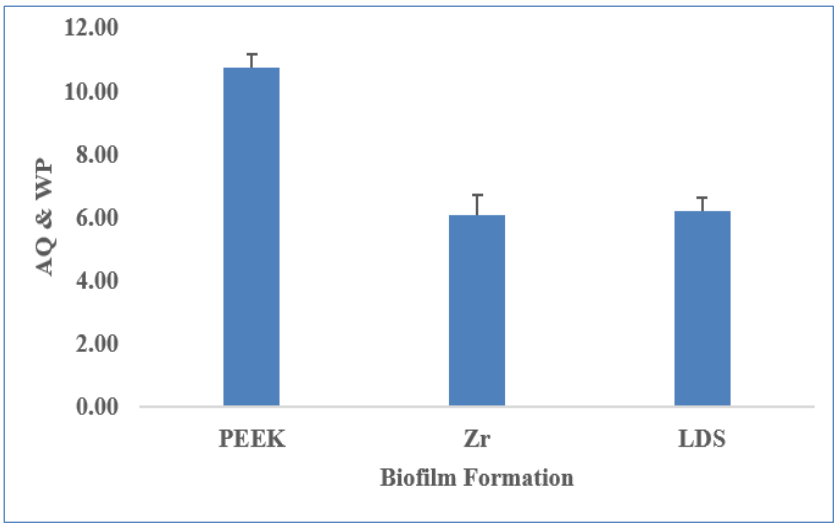


Figure 4.21: Comparison of the mean values of ΔE* between all three materials after biofilm formation and after cleaning with AQ and WP

There were statistically significant differences between PEEK and ZrO_2 (Tukey test, $p \leq 0.05$), PEEK and LDS (Tukey test, $p < 0.05$). However, no statistically significant difference between the mean values of ΔE* for ZrO_2 and LDS (Tukey test $p > 0.05$) (Table 4.24).

Table 4.24: Comparison of the mean values of ΔE* between each pair of the three materials after biofilm formation and after cleaning with AQ and WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
ΔE* Biofilm formation vs (AQ& WP)	PEEK	ZrO_2	4.65	0.72	2.91	6.40	0.000
		LDS	4.52	0.72	2.77	6.27	0.000
	ZrO_2	PEEK	-4.65	0.72	-6.40	-2.91	0.000
		LDS	-0.13	0.72	-1.87	1.61	0.981
	LDS	PEEK	-4.52	0.72	-6.27	-2.77	0.000
		ZrO_2	0.13	0.72	-1.61	1.87	0.981

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$

4.5.2 Unpolished Surfaces

The mean values of ΔE* for PEEK after biofilm formation and after cleaning with AQ and WP was measured and compared. PEEK showed the highest mean value of 10.33, followed by ZrO_2 with mean value of 7.29 and LDS with the lowest mean value of 6.14 (Figure 4.22).

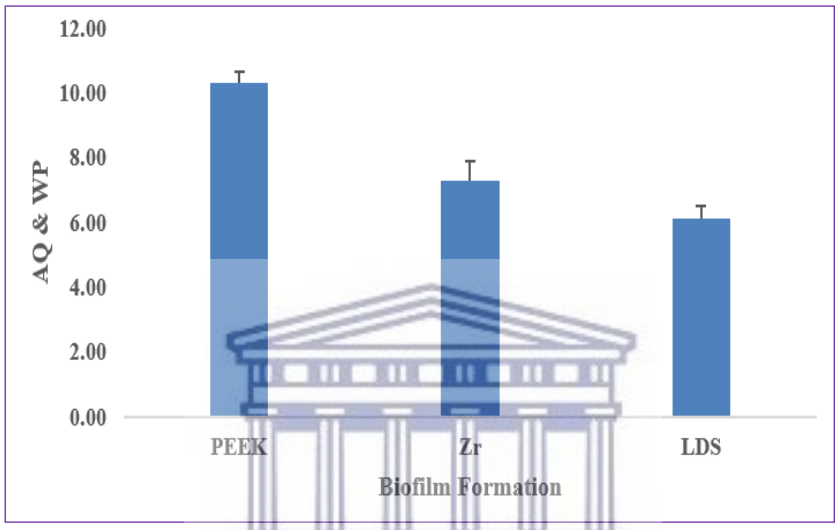


Figure 4.22: Comparison of the mean values of ΔE* between all three materials after biofilm formation and after cleaning with AQ and WP

There were statistically significant differences between PEEK and ZrO_2 (Tukey test, $p \leq 0.05$), PEEK and LDS (Tukey test, $p \leq 0.05$). However, no statistically significant difference was found between the mean values of ΔE* for ZrO_2 and LDS (Tukey test, $p > 0.05$) (Table 4.25).

Table 4.25: Comparison of the mean values of ΔE* between each pair of the three materials after biofilm formation and after cleaning with AQ and WP

Dependent Variable			Mean Difference (I-J)	95% Confidence Interval		P value
				Lower	Upper	
ΔE* Biofilm formation vs (AQ& WP)	PEEK	ZrO_2	3.038	1.51	4.56	0.000
		LDS	4.18	2.66	5.72	0.000
	ZrO_2	PEEK	-3.04	-4.56	-1.51	0.000
		LDS	1.15	-0.37	2.68	0.173
	LDS	PEEK	-4.19	-5.72	-2.66	0.000
		ZrO_2	-1.15	-2.68	0.37	0.173

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$

4.6 Efficacy of the Pulsating Devices (Aquaflosser & Waterpik) to Remove Biofilm from the Surfaces of PEEK, ZrO_2 and LDS

The efficacy of the two pulsating devices to remove biofilm was determined by calculating the mean values of ΔE* for all three materials at baseline and after cleaning with the two pulsating devices (AQ and WP).

4.6.1 Polished Surfaces

The mean values of ΔE* were compared for all three materials at baseline and after cleaning with the two pulsating devices (AQ and WP). There were statistically significant differences (ANOVA $p \leq 0.05$) between all there materials. PEEK showed the highest mean value of 2.05, followed by LDS with mean value of 0.95 and ZrO_2 with the lowest mean value colour change 0.57 (Figure 4.23).

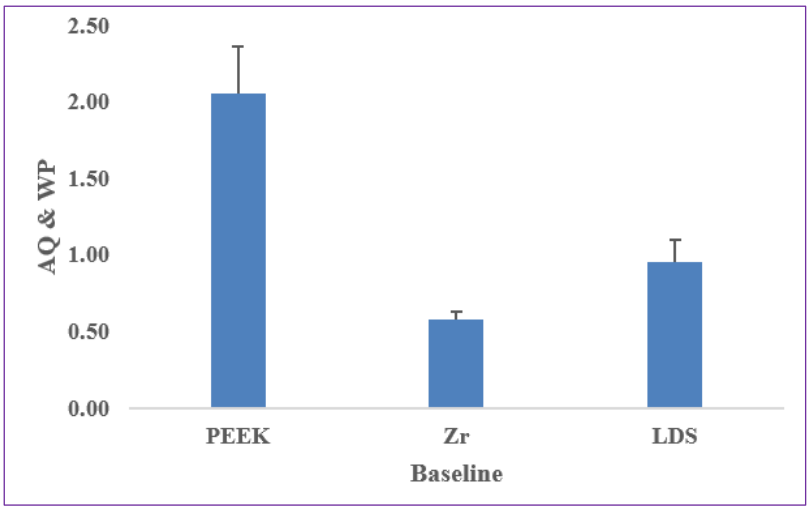


Figure 4.23: Comparison of the mean values of ΔE* for all three materials at baseline and after cleaning with AQ & WP

There were statistically significant differences between PEEK and ZrO_2 (Tukey test, $p \leq 0.05$), PEEK and LDS (Tukey test, $p \leq 0.05$). However, no statistically significant difference was found between the mean values of ΔE* for ZrO_2 and LDS (Tukey test, $p > 0.05$) (Table 4.26).

Table 4.26: Comparison of the mean values of ΔE* between each pair of the three materials at baseline and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
ΔE* Baseline vs (AQ& WP)	PEEK	ZrO_2	1.47	0.28	0.79	2.15	0.000
		LDS	1.09	0.28	0.41	1.77	0.000
	ZrO_2	PEEK	-1.47	0.28	-2.15	-0.79	0.000
		LDS	-0.37	0.28	-1.05	0.30	0.376
	LDS	PEEK	-1.09	0.28	-1.77	-0.41	0.000
		ZrO_2	0.37	0.28	-0.30	1.05	0.376

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$

4.6.2 Unpolished Surfaces

The mean values of ΔE* between all three materials at baseline and after cleaning with the two pulsating devices (AQ & WP) was compared. There were statistically significant differences (ANOVA $p \leq 0.05$) between all there materials. PEEK had the highest mean value of ΔE* 2.52, followed by LDS with mean value of 0.88 and ZrO_2 0.83 with the lowest value colour change (Figure 4.24).

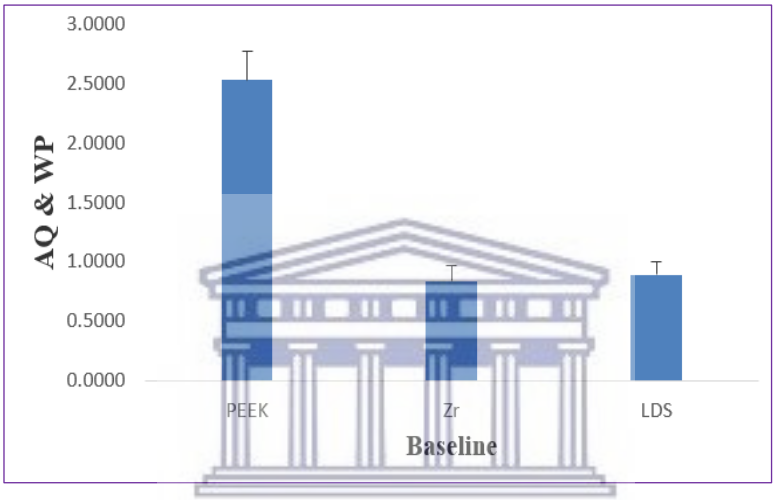


Figure 4.24: Comparison of the mean values of ΔE* for all three materials at baseline and after cleaning with AQ & WP

There was statistically significant difference between PEEK and ZrO_2 (Tukey test, $p \leq 0.05$), PEEK and LDS (Tukey test, $p \leq 0.05$), but no statistically significant difference was found between the mean values of ΔE* for ZrO_2 and LDS (Tukey test, $p > 0.05$) (Table 4.27).

Table 4.27: Comparison of the mean values of ΔE* between each pair of the three materials at baseline and after cleaning with AQ & WP

Dependent Variable			Mean Difference (I-J)	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
ΔE* Baseline vs (AQ& WP)	PEEK	ZrO_2	1.69*	0.24	1.10	2.28	0.000
		LDS	1.63*	0.24	1.04	2.23	0.000
	ZrO_2	PEEK	-1.69*	0.24	-2.28	-1.10	0.000
		LDS	-.053	0.24	-0.64	0.53	0.973
	LDS	PEEK	-1.63*	0.24	-2.23	-1.04	0.000
		ZrO_2	.054	0.24	-0.53	0.64	0.973

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$

4.7 Efficacy of the Two Pulsating Devices Aquaflosser & Waterpik on Both Polished and Unpolished Surfaces for All Three Materials

To determine which of the two pulsating devices can remove biofilm from the polished and unpolished surfaces more efficiently, the mean values of ΔE* were compared between the two pulsating devices (AQ and WP) with the materials after biofilm formation and the materials at baseline respectively. There were no statistically significant differences (ANOVA $p<0.05$) when comparing the mean values of ΔE* for both polished and unpolished surfaces for all three materials at baseline and after biofilm formation with the materials after cleaning with the two pulsating devices (Table 4.28).

Table 4.28: Comparison of the mean values of ΔE* between all three materials (polished & unpolished) after cleaning with AQ and WP with the materials after biofilm formation and at baseline respectively

Surface			Mean	Std. Error Mean	P value
Polished	ΔE* Biofilm formation vs (AQ & WP)	AQ	7.80	0.58	0.708
		WP	7.49	0.60	
	ΔE* Baseline vs (AQ & WP)	AQ	1.43	0.24	0.089
		WP	0.95	0.12	
Unpolished	ΔE* Biofilm formation vs (AQ & WP)	AQ	8.07	0.52	0.671
		WP	7.77	0.47	
	ΔE* Baseline vs (AQ & WP)	AQ	1.61	0.22	0.188
		WP	1.22	0.18	

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$

4.8 Assessment of the Efficacy of Aquaflosser and Waterpik with Each of the Three Materials

To compare the cleaning efficacy of both devices, the mean values of ΔE* were compared between all three materials at baseline and after biofilm formation with the materials after cleaning with the two pulsating devices (AQ and WP).

4.8.1 Polished Surfaces

4.8.1.1 Comparison Between Aquaflosser and Waterpik with Each of the Three Materials at Baseline

AQ showed the highest mean values of 2.59 with PEEK followed by LDS with a mean value of 1.06 and ZrO_2 with the lowest mean value of 0.66. Whereas WP showed the highest mean values of 1.51 with PEEK followed by LDS with a mean value of 0.85 and ZrO_2 with the lowest mean value of 0.49 (Figure 4.25; Table 4.29).

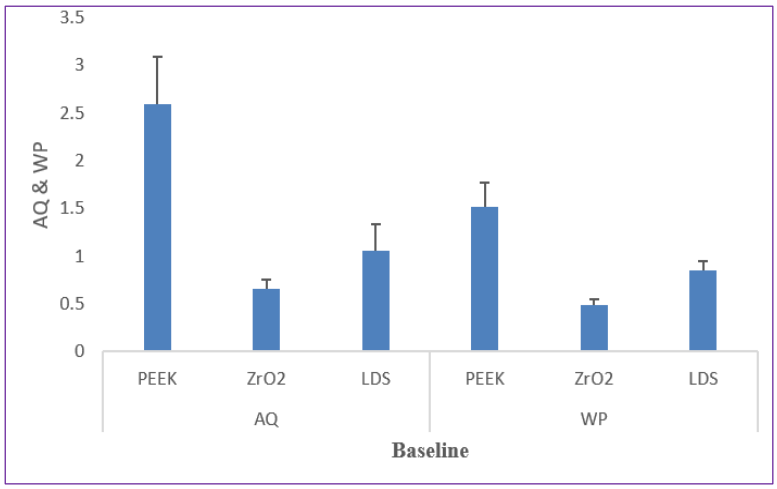


Figure 4.25: Comparison of the mean values of ΔE* between all three materials at baseline with the materials after cleaning with AQ & WP

Table 4.29: Comparison of the mean values of ΔE* between all three materials at baseline with the materials after cleaning with AQ & WP

Device			Mean	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
AQ	ΔE* Baseline vs (AQ & WP)	PEEK	2.59	0.50	1.41	3.76	0.001
		ZrO_2	0.66	0.09	0.44	0.88	
		LDS	1.06	0.27	0.43	1.69	
WP	ΔE* Baseline vs (AQ & WP)	PEEK	1.51	0.26	0.90	2.13	0.000
		ZrO_2	0.49	0.06	0.34	0.63	
		LDS	0.85	0.10	0.60	1.09	

Differences statistically significant at p≤0.05; Statistically not significant p>0.05

4.8.1.2 Comparison Between Aquaflosser and Waterpik with Each of the Three Materials After Biofilm Formation

AQ showed the highest mean value of 10.32 with PEEK followed by ZrO_2 with a mean value of 6.93 and the lowest mean values of 6.16 with LDS. Whereas WP showed the highest mean values of 11.09 with PEEK followed by LDS with a mean value of 6.21 and the lowest mean value of 5.17 with ZrO_2 (Figure 4.26; Table 4.30).

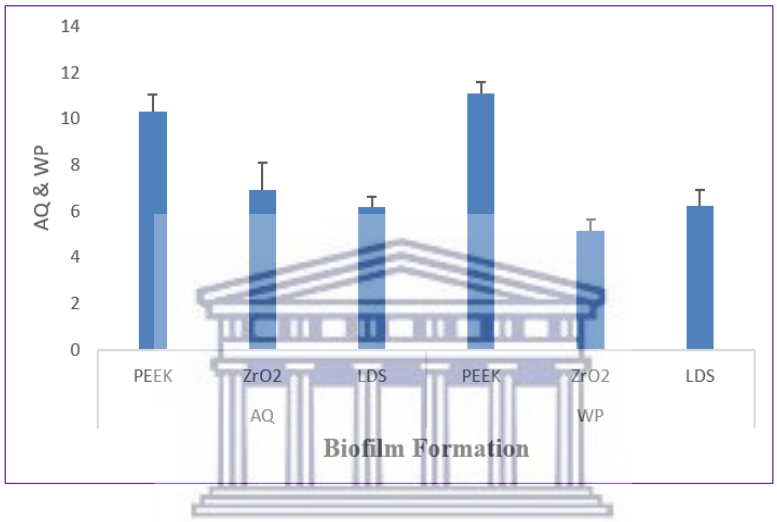


Figure 4.26: Comparison of the mean values of ΔE* between all three materials after biofilm formation with the materials after cleaning with AQ & WP

Table 4.30: Comparison of the mean values of ΔE* between all three materials after biofilm formation with the materials after cleaning with AQ & WP

Device			Mean	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
AQ	ΔE* Biofilm formation vs (AQ & WP)	PEEK	10.32	0.72	8.65	11.99	0.003
		ZrO_2	6.93	1.16	4.25	9.62	
		LDS	6.16	0.44	5.13	7.19	
WP	ΔE* Biofilm formation Vs (AQ & WP)	PEEK	11.09	0.51	9.90	12.28	0.000
		ZrO_2	5.17	0.46	4.09	6.25	
		LDS	6.21	0.73	4.51	7.90	

4.8.2 Unpolished Surfaces

4.8.2.1 Comparison Between Aquaflosser and Waterpik with Each of the Three Materials at Baseline

AQ showed the highest mean values of 2.78 with PEEK followed by ZrO_2 with mean value of 1.21 and LDS with lowest mean value of 0.84. Whereas WP showed the highest mean value of 2.27 with PEEK followed by LDS with a mean value of 0.94 and ZrO_2 with the lowest mean value of 0.46 (Figure 4.27; Table 4.31).

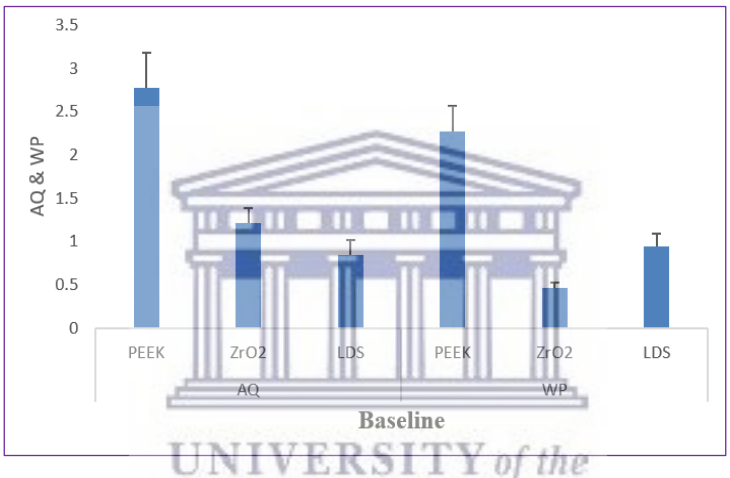


Figure 4.27: Comparison of the mean values of ΔE* between all three materials at baseline with the materials after cleaning with AQ & WP

Table 4.31: Comparison of the mean values of ΔE* between all three materials at baseline with the materials after cleaning with AQ & WP

Device			Mean	Std. Error	95% Confidence Interval		P value
					Lower	Upper	
AQ	ΔE* Baseline vs (AQ & WP)	PEEK	2.78	0.40	1.86	3.71	0.000
		ZrO_2	1.21	0.18	0.79	1.62	
		LDS	0.84	0.17	0.45	1.24	
WP	ΔE* Baseline vs (AQ & WP)	PEEK	2.27	0.29	1.61	2.93	0.000
		ZrO_2	0.46	0.07	0.30	0.62	
		LDS	0.94	0.15	0.58	1.29	

4.8.2.2 Comparison Between Aquaflosser and Waterpik with Each of the Three Materials After Biofilm Formation

AQ showed the highest mean value of 10.06 with PEEK followed by ZrO_2 with a mean value of 8.12 and LDS with the lowest mean values of 6.04. Whereas WP showed the highest mean values of 10.61 with PEEK followed by ZrO_2 with a mean value of 6.47and LDS with the lowest mean value of 6.24 (Figure 4.28; Table 4.32).

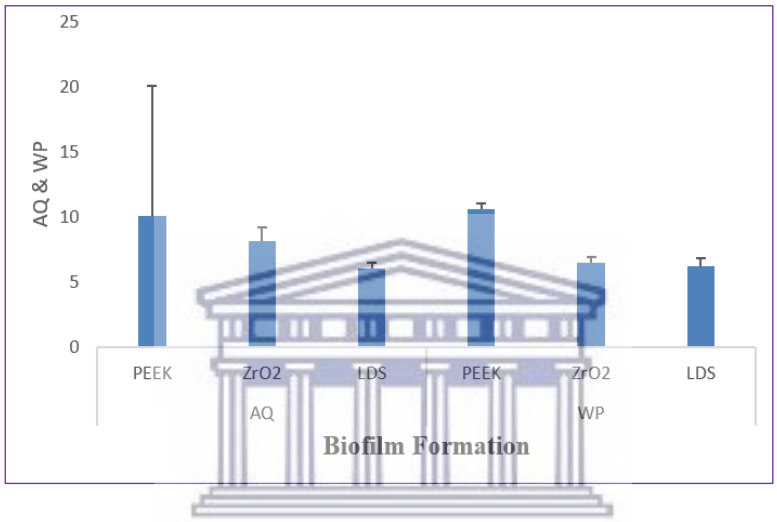


Figure 4.28: Comparison of the mean values of ΔE* between all three materials after biofilm formation with the materials after cleaning with AQ & WP

Table 4.32: Comparison of the mean values of ΔE* between all three materials after biofilm formation with the materials after cleaning with AQ & WP

Device			Mean	Std. Error	95% Confidence Interval for Mean		P value
					Lower	Upper	
AQ	ΔE* Biofilm formation vs (AQ & WP)	PEEK	10.06	10.06	10.06	10,06	0.003
		ZrO_2	8.12	1.08	5.62	10,06	
		LDS	6.04	0.43	5.04	7.05	
WP	ΔE* Biofilm formation Vs (AQ & WP)	PEEK	10.61	0.42	9.65	11.57	0.000
		ZrO_2	6.47	0.44	5.45	7.48	
		LDS	6.24	0.59	4.88	7.60	

Differences statistically significant at $p \leq 0.05$; Statistically not significant $p > 0.05$

4.9 Scanning Electron Microscopy (SEM) Results

For all materials analyzed, SEM images revealed that the surfaces which were unpolished had more irregular surface topography than the respective polished surfaces (Figure 4.29). Parallel scratch marks and small pits were more often seen on polished surfaces, which were consistent with normal preparation and polishing artefacts, whereas unpolished surfaces displayed coarse pits and irregularities, as evidenced by a rougher surface.

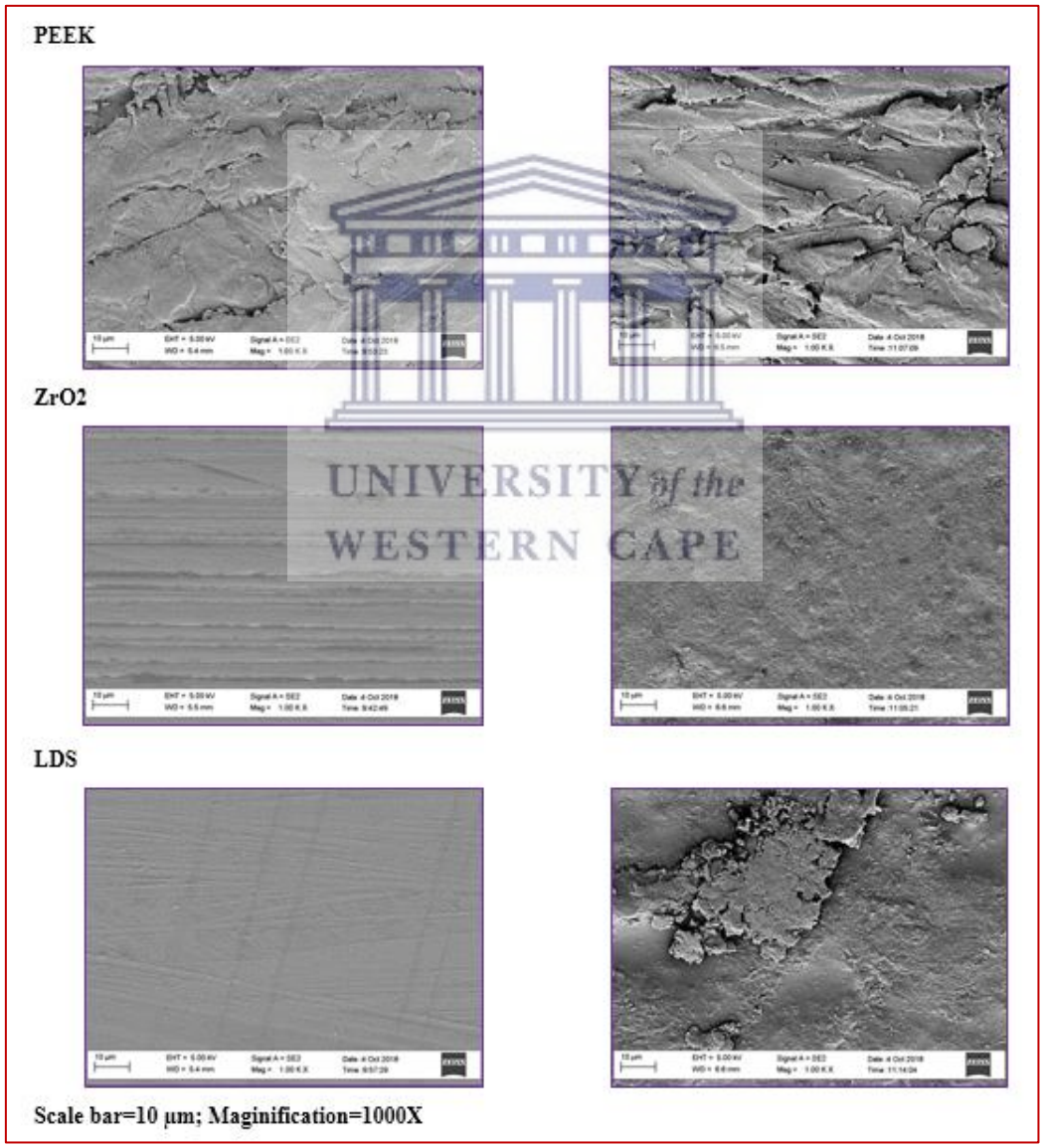


Figure 4.29: SEM images of polished and unpolished surfaces of the materials

The polished surface of PEEK exhibited the roughest surface (Figure 4.30), LDS recorded smoother polished surface (Figure 4.32), whereas ZrO_2 recorded the smoothest polished surface with relatively small pits and scratches (Figure 4.31).

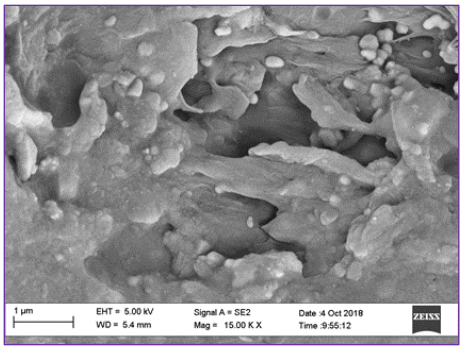


Figure 4.30: PEEK polished surface

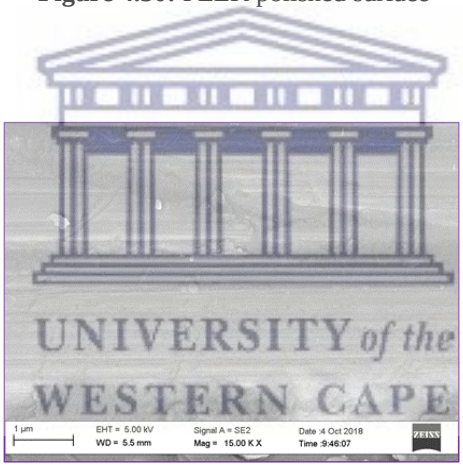


Figure 4.31: ZrO_2 polished surface

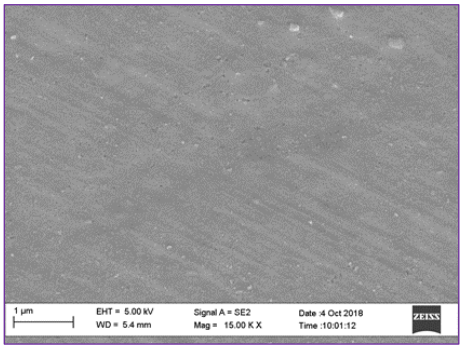
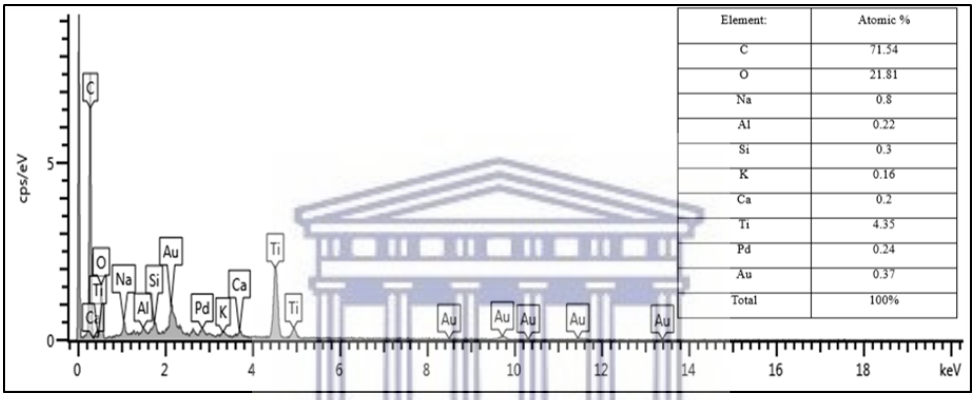


Figure 4.32: LDS polished surface

4.10 Energy Dispersive Spectroscopy (EDS)

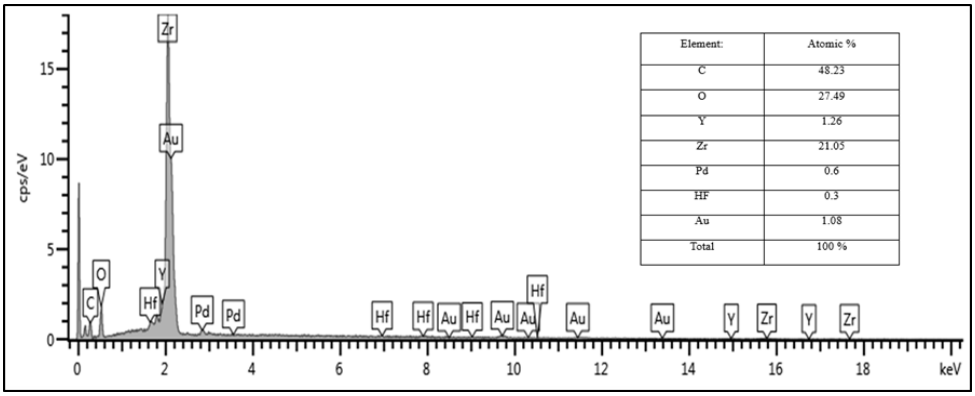
Mapping with EDS was carried out to characterize regions corresponding to ceramic and polymer. EDS relies on an interaction of the source of X-ray excitation and a sample. Its characterization capabilities are due to the fundamental principle that each element has a unique atomic structure allowing a unique set of peaks on its electromagnetic emission spectrum. (Figure 4.33) shows the elemental composition of PEEK.



Element:	Atomic %
C	71.54
O	21.81
Na	0.8
Al	0.22
Si	0.3
K	0.16
Ca	0.2
Ti	4.35
Pd	0.24
Au	0.37
Total	100%

Figure 4.33: Elemental composition of PEEK

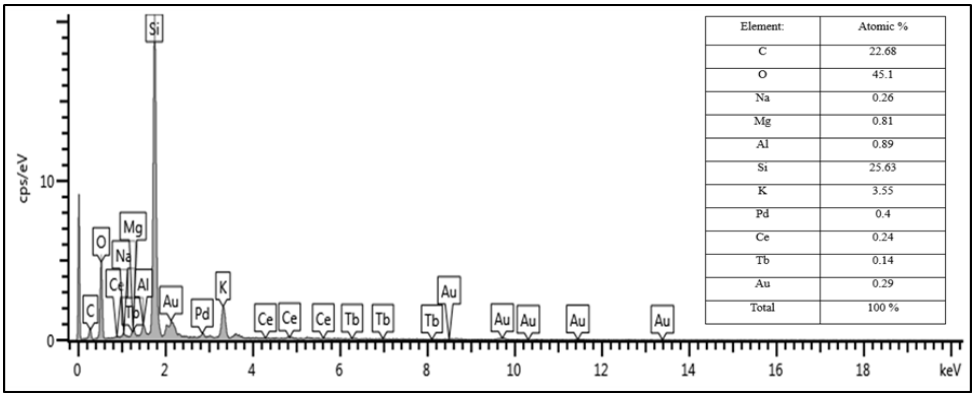
Elemental composition of ZrO_2 can be confirmed by the presence of yttrium and zircon (Figure 4.34).



Element:	Atomic %
C	48.23
O	27.49
Y	1.26
Zr	21.05
Pd	0.6
HF	0.3
Au	1.08
Total	100 %

Figure 4.34: Elemental composition of ZrO_2

Elemental composition of LDS is confirmed by the presence of silicate (Figure 4.35).



Element:	Atomic %
C	22.68
O	45.1
Na	0.26
Mg	0.81
Al	0.89
Si	25.63
K	3.55
Pd	0.4
Ce	0.24
Tb	0.14
Au	0.29
Total	100 %

Figure 4.35: Elemental composition of LDS

CHAPTER 5

DISCUSSION

5.1 Introduction

Knowledge about the adherence of bacteria to different materials is of crucial importance to achieve long-term success of dental restorations. Polymeric materials generally accumulate more plaque on their surface than alloys or ceramics (Sakaguchi, *et al.*, 2018). The surface properties of a material have a pronounced influence particularly on the early phases of biofilm formation (Hahnel *et al.*, 2015). It has been reported that the surface roughness (Ra) and surface free energy (SFE) are among the factors that have been identified to influence oral biofilm formation, suggesting that smooth surfaces and those with low SFE display less microbial adherence than materials with higher surface roughness or SFE (Hahnel *et al.*, 2015).

Increased surface roughness and complicated topography shows higher affinity to microbes than smoother surfaces and subsequently increased difficulty in complete removal of the biofilm by mechanical brushing (Aykent *et al.*, 2010). Thus, smoothening the surface can reduce biofilm formation (Ionescu *et al.*, 2012). A maximum surface roughness of Ra-0.2 µm has been suggested as a threshold value for bacterial retention (Jalalian *et al.*, 2014). Below this value, no further reductions were observed, while above this value biofilm accumulation increased with increasing roughness (Jalalian *et al.*, 2014). Furthermore, Rashid (2014) reported that rougher surfaces of crowns, bridges and implant abutments accumulate and retain more plaque.

Viitaniemi *et al.*, (2017) stated that highly polished surfaces facilitate effective biofilm removal from prosthetic structures, but all subgingival surfaces and structural areas, which are difficult to clean, are potential bacterial colonization sites. The results of the present study are in accordance with previous studies were biofilm formation was generally to be found higher on the unpolished materials and relatively less on the polished materials (Teughels *et al.*, 2006).

On the other hand, Meier *et al*., (2008) found that increasing the Ra value of several dental ceramics five-fold did not result in a significantly higher number of adherent bacteria. This result is in agreement with the results acquired in the present study where there was no statistical significant difference in biofilm formation between polished and unpolished surfaces in Lithium disilicate and Zirconium whereas for PEEK there was a difference. In addition, it has been suggested that bacterial adhesion is influenced largely by nanometer-scale changes of surface roughness concluding that nanoscale changes maybe more sensitive to bacterial adhesion than changes on a macro-scale level (Mitik-Dineva *et al.*, 2008).

Etxeberria *et al.*, (2013) claimed that surface roughness and wettability are strongly correlated with bacterial adhesion, suggesting that the bacterial adhesion is not influenced by roughness alone. Thus, to date no consensus has been obtained in the literature on the role of surface roughness on bacterial attachment. The chemical composition of dental materials will further affect the bacterial adhesion, since both proteins and microorganisms can chemically attach or attract components in the material by means of Van der Waal forces, acid-base reactions or electrostatic interactions (Øilo & Bakken, 2015).

Yu *et al.,* (2016) demonstrated the effect of polishing zirconia blocks where it was polished on three different levels; coarse, medium and fine using carbide burs. Using the acridine stain, coarse samples had the largest number of adherent bacteria compared to the medium and fine samples, indicating that there is a correlation between the surface roughness and the amount of colonized bacteria (Yu *et al.*, 2016). Samples in the present study were incubated for 6 days while Yu *et al.,* (2016) incubated the samples for 24hours only giving the bacteria less time to colonize the discs. It was also noted that during the 4-12 hours incubation period the highest amount of bacteria was recorded, thereafter the amount decreased. This reflect that the correlation was positive in the period from 4-12 hours whereas a negative correlation was observed at 24 hours (Yu *et al.*, 2016). Carbide burs also exert more surface roughness when compared to finishing discs used in the present study (Yu *et al.*, 2016).

5.2 Evaluation of Biofilm Formation on Zirconium, Lithium Disilicate & PEEK

The results of this *in-vitro* study reject the first research hypothesis suggesting that there is no difference on biofilm formation among all three materials namely Zirconia, Lithium disilicate and PEEK. The biofilm formation achieved in the present study showed that there was a colour difference (ΔE*) between all three materials. PEEK showed the biggest change with most biofilm accumulation as indicated by a decrease in L* & b* and increase in a* scale where L* stands for lightness (black-white), a* red-green coordinate and b* yellow-blue coordinate.

The mean values of L* for the polished surfaces of PEEK decreased from 88.61 at baseline to 82.98 after biofilm formation, the b* decreased from 4.46 at baseline to -3.25 after biofilm formation, whereas the a* increased from 0.52 at baseline to 8.58 after biofilm formation. For polished surfaces of LDS the mean value of L* decreased from 71.29 at baseline to 68.34 after biofilm formation, the b* decreased from 18.71 to

14.68, while a mean value increased from 3.78 at baseline to 8.45 after biofilm formation was observed on a* scale. Whereas, the mean value of L* for polished surfaces of ZrO_2 decreased from 77.29 at baseline to 74.48 after biofilm formation, the b* also decreased from 11.73 at baseline to 8.96 after biofilm formation. For a* the mean value increased from 1.17 at baseline to 6.13 after biofilm formation. These results were further confirmed by calculation of ΔE* where the PEEK showed the highest mean value of 12.63 followed by LDS with a mean value of 6.99 and ZrO_2, with the lowest mean value of 6.31. It can therefore be concluded that ZrO_2 is more superior than the other two materials.

This indicates that PEEK material attracts more biofilm formation followed by LDS and ZrO_2. This may be due to machined PEEK having a surface roughness with non-uniform features compared to LDS and ZrO_2. The results of this study were similar to the study by Øilo & Bakken (2015) which stated that Ceramic materials have a smooth, polished surface that are easily cleaned, in contrast to polymers that develop biofilm quicker and will be more difficult to remove completely. SEM pictures taken in the present study further illustrate the point where PEEK showed the roughest surface (Figure 4.30).

Moreover, Gorth *et al.*, (2012) examined two surface finishes of silicon nitride (Si_3N_4) with titanium (Ti) and PEEK. Ti and PEEK were received as machined surfaces: both materials are hydrophobic with net negative surface charges. Two surface finishes of Si_3N_4 were examined, both fired and polished. In contrast to Ti and PEEK, the surface of Si_3N4 is hydrophilic with a net positive charge. A decreased biofilm formation was found, as well as fewer live bacteria on both the fired and polished Si_3N_4.

Hydrophilic Si_3N_4 surfaces were probably less conducive to bacterial adhesion, when

compared with hydrophobic surfaces where water displacement is not required for microbial adherence. These differences may reflect differential surface chemistry and surface nanostructure properties between the biomaterials tested (Gorth *et al*, 2012). As protein adsorption on material surfaces affects bacterial adhesion, the adsorption of fibronectin, vitronectin, and laminin on Ti, PEEK, and Si_3N_4 were also examined (Gorth *et al*, 2012) Significantly greater amounts of these proteins adhered to Si_3N_4 than to Ti or PEEK. These findings suggest that surface properties of biomaterials lead to differential adsorption of physiologic proteins, and that this phenomenon could explain the observed *in-vitro* differences in bacterial affinity for the respective biomaterials

In addition, laboratory studies have shown that biofilms can be reproducibly grown on the surface of PEEK (Williams *et al.*, 2011), where a modified CDC biofilm reactor was developed to repeatable grow mature biofilms of *Staphylococcus aureus* on the surface of PEEK membranes for inoculation in a future animal model. Results indicated that uniform, mature biofilms repeatedly grew on the surface of PEEK membranes. Furthermore, Rochford *et al*., (2016) compared the adhesion of bacteria to two surfaces of PEEK (machined surface and injection molding of PEEK) with standard micro rough implant grade titanium surfaces. Although the exact results differed between strains, the propensity for bacteria to adhere to injection-molded PEEK was similar to micro rough titanium *in vitro* (Rochford *et al*, 2016).

In addition, bacterial adhesion to machined PEEK was generally higher than to titanium despite having similar roughness values. This illustrates the significance of the specific topography of machined PEEK for promoting bacterial adhesion. This was attributed to the fact that machining of PEEK results in relatively rough surface with no uniform features, while injection molding of PEEK produces a relatively smooth topography (a

reflection of the mold) with minimal plateaus and ridges. The results acquired from the research comparing PEEK to titanium supports the superiority of Zirconia to PEEK because several other studies concluded that Zirconia has a lower number of adherent bacteria when compared to titanium (Roehling *et al*, 2017). These findings support the results of the present study, which found that Zirconia has lower number of adherent bacteria when compared to PEEK.

To date limited scientific evidence is available regarding biofilm formation on abutments made from PEEK. The study investigated microbial issues on implants supplied with PEEK abutments identified similar microbial counts and levels of periodontal pathogens in the peri-abutment region of implants supplied with PEEK and titanium healing abutments, when evaluated using real-time PCR (Volpe *et al*., 2008).

Similarly, a laboratory study investigating the formation of biofilms on the surface of titanium, Zirconia, PMMA and PEEK showed almost similar biofilm formation on the various materials (Hahnel *et al*., 2015). However, it is doubtful whether the differences in surface roughness values identified for the materials investigated can account for differences in biofilm formation, although the surface roughness of titanium and Zirconia was significantly higher than the surface roughness of both PEEK and PMMA. Biofilm formation did not correlate with these results as significantly more viable biomass was identified on PMMA than on PEEK.

Recent studies suggest that the surface composition and surface topography might impact the formation of biofilms to an even higher level (Ionescu *et al*., 2012; Hahnel *et al*., 2014), which might serve as an explanation for the poor correlation between surface properties and biofilm formation observed in the study by Hahnel *et al*., (2015). These findings are supported by the results of a clinical study analyzing the bacterial

colonization of healing abutments made from PEEK and titanium, where no significant differences between PEEK and titanium could be identified. However, the data of that study needs to be interpreted with caution, as only small samples of the biofilm adherent to the surfaces were analyzed by employing the real-time PCR technique (Volpe *et al*., 2008).

Regarding biofilm formation on the surfaces of Zirconia and LDS, the current study showed that biofilm is formed more readily on LDS surfaces than on ZrO_2 although there was no statistically significant difference. This can be attributed to the elongated LDS crystals. This result is in accordance with Hahnel *et al.*, (2009) and Guazzato *et al.*, (2004), where they assessed different dental ceramic classes and initial *Streptococci* adhesion. They found that the crystalline content of the ceramic substrata showed no correlation with Ra. Zirconia ceramics possess homogeneous grains with an average grain size of about 0.3-m, which corresponds to the intermediate values for Ra found for the partially stabilized zirconia and zirconia processed with hot isotonic pressing (hipped) used in the study. LDS ceramic displayed the highest Ra values; this trend was significant and is most likely due to its elongated lithium disilicate crystals. They concluded that dental ceramic classes differ significantly in terms of surface roughness and surface free energy.

Bremer *et al.,* (2010) further confirmed these results; they reported that the lowest values for biofilm surface coating and biofilm thickness were found for zirconia ceramic when compared with other types of ceramics. They concluded that zirconia ceramics are advantageous compared to LDS glass ceramics that meet similar indications. The adhesion of bacteria is not related to the different topographical surface characteristics, but appears to be linked to the chemical composition, the crystallographic architecture, or the surface free energy of ceramics.

Rimondini *et al.,* (2002) analyzed the adhesion and inhibition of oral microorganism's growth *in vitro* on zirconia compared to titanium. They concluded that adhesion differences could be observed for some of the selected microorganisms. Scanning electron microscopy analysis revealed a lower biofilm accumulation on zirconia than on titanium (Rimondini *et al*., 2002). Scarano *et al.,* (2005) showed that the onset of adhesion or bacterial colonization on zirconia surfaces was significantly reduced when compared to titanium.

Furthermore, Jalalian *et al.,* (2015) evaluated adhesion of *S. mutans* on zirconia, Feldspatic porcelain, titanium alloy and indirect composite. Evaluated materials showed different surface roughness; however, all of them had roughness values lower than 1 µm which is considered clinically very smooth. Zirconia showed the lowest bacterial adhesion in comparison to other tested materials and enamel. No correlation was found between surface roughness and bacterial adhesion as well as other factors such as hydrophobicity or surface free energy of bacterium or oral surfaces, the ionic strength of the surrounding liquid medium and electrostatic interactions.

On the contrary, a study by Viitanimi *et al.,* (2017), which examined adhesion and early colonization of *S. mutans* on LDS, ZrO_2 and Dual cure resin cement. In this study, highly polished surfaces of the materials were used to indicate lower surface roughness values (Ra), the results showed that LDS glass ceramic possessed lower bacterial adhesion than either fully or partially stabilized ZrO_2 materials and they concluded that the lowest hydrophobicity and highest surface free energy influence adhesion of *S. mutans*.

As for colour stability, Volpato *et al.,* (2016) evaluated the effect of different aging times on the zirconia whether veneered or not, using a spectrophotometer. The

specimens were subjected to an accelerated aging protocol for different durations. The colour difference recorded was less than 1.25 ΔE* indicating that zirconia maintains its colorimetric properties. Moreover, Palla *et al.,* (2018) investigated the colour stability of LDS after aging and immersion in common beverages. They found that the colour change was significantly low and below the clinical perception. CAD LDS showed a high colour stability.

Concerning colour changes, a study was done by Heimer *et al.*, (2017), on discoloration of PEEK compared to composite and PMMA. The samples were stored in three different media including distilled water, wine and curry. The research results showed that PEEK was the most stable of the three materials used. This result is in accordance with the results of this study confirming that the disclosing agent has no effect on colour changing and that biofilm formation is the main cause of the colour change, as the disclosing agent acts as an indicator by reacting with the biofilm and causing the colour change.

5.3 Evaluation of Pulsating Devices to Remove Biofilm from Three Materials

Oral irrigating devices have two main features that allow them to remove dental plaque or biofilms easily from the surfaces, namely pulsation and pressure. This combination provides for phases of compression and decompression of the tissue to help expel subgingival bacteria and other debris, as well as stimulate gingival tissue (John, 2010). Studies have shown that a pulsating device was 3 times more effective than a continuous stream device (John, 2010). Thus, these two features allow the devices to disrupt the bacterial activity along with expulsion of subgingival bacteria and removal of food debris

The oral devices used in the present study showed no significant difference in the

cleaning efficiency between Aquaflosser and Waterpik to remove biofilm from PEEK, LDS and ZrO_2, thus the null hypothesis for the second part of the study was failed to be accepted. This was indicated by AQ with the highest mean value of 10.32 with PEEK followed by ZrO_2 with a mean value of 6.93 and lowest mean values of 6.16 with LDS. Whereas WP showed the highest mean values of 11.09 with PEEK followed by LDS with a mean value of 6.21 and lowest mean value of 5.17 with ZrO_2.

The results of this study can be attributed to the fact that both devices used the same pressure during cleaning. Several studies were conducted to compare Waterpik and interdental brushing or manual brushing in general, Waterpik was found to be more effective than interdental brushing in removing dental biofilms from the various tooth surfaces as well as reducing gingival bleeding and probing depth (Lyle *et al.*, 2016).

Rosema *et al.,* (2011) compared effectiveness of Waterpik to that of a Prototype jet tip as an adjunct to daily tooth brushing. They concluded that when combined with tooth brushing, Waterpik was very effective in reducing gingival scores.

Gorur *et al.,* (2008) incubated eight slices obtained from four teeth extracted from a patient suffering from advanced aggressive periodontitis in saliva taken from a volunteer and another four teeth were not incubated in saliva (control). Four slices were treated using a dental water jet with a standard jet tip and the other four were treated using an orthodontic jet tip for 3 sec in medium pressure. SEM showed that slices that are treated using the standard tip removed 99.99% of the salivary biofilm while the orthodontic jet tip removed 99.84%.

Howlin *et al.,* (2015) compared the use of water stream only with an ultrasonically activated water stream to remove biofilms of *S. mutans* from petri dishes. They found

that exposure to a 10s ultrasonically activated water stream removed 99% of the *S. mutans* biofilms suggesting oral irrigating devices efficiency in removing dental biofilms. Another study supporting this result, by Tawakoli *et al.*, (2015) compared oral irrigating devices' and sonic toothbrush's efficiency in removing biofilms of *S. mutans* cultured on hydroxyapatite blocks *in vitro*. Oral irrigating devices namely Waterpik recorded the highest reduction in metabolic activity of the adherent bacteria. Under SEM, the specimen barely revealed any bacteria.

Orthodontists developed an interest in oral irrigating devices as a mean of plaque removal, which is one of the challenges they face in maintaining an optimum oral hygiene environment for the patient. Patel *et al.*, (2015) gathered sixty orthodontic patients with fixed appliances and divided them into four groups. Each group was assigned to a method for removing plaque, with and without water irrigating devices, and gingival index as a measurement. After one month, the gingival indexes of the oral irrigating devices showed better gingival index scores when compared to the other groups using, manual toothbrushes, automatic toothbrushes and both.

However, since Aquaflosser has been introduced to the market recently, no literature has been published regarding its efficacy.

CHAPTER 6

CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

6.1 Conclusions

This *in vitro* study that investigated the ability of biofilm to form on three fixed prosthodontic materials (PEEK, Zirconium & Lithium disilicate) and the ability of two pulsating devices (Aquaflosser and Waterpik) to remove biofilm from these three materials. Based on the results, the first null hypothesis was rejected, and the second null hypothesis was failed to be rejected.

Within the limitation of this study, it can be concluded that:

- PEEK showed the highest biofilm formation among the materials tested.
- Lithium disilicate showed higher biofilm formation than Zirconia but lower than PEEK.
- Zirconia showed the lowest biofilm formation in all parameters. However, there was no statistically significant difference when compared to Lithium disilicate.
- Although both pulsating devices (Aquaflosser & Waterpik) are efficient to

remove biofilm from the polished and unpolished surfaces of the material, there was no significant difference between the two devices when compared.

6.2 Limitations of the Study

- The samples used in the study were not pre-coated with saliva that may differ from the clinical situation where the materials will be coated with saliva.

- This study was conducted under ideal laboratory conditions where the pulsating devices can reach all surfaces of the samples, which may not be possible *in vivo* as the anatomy of the tooth and its position on the oral cavity plays a role in affecting the cleaning efficacy of pulsating devices. Recommendations:

6.3 Recommendations

- In the current study, biofilm formation was evaluated using spectrophotometric analysis. A further study that uses direct microscopic methods (i.e. confocal electron microscopy) may be needed to add value to the present study.

- Aquaflosser has recently been introduced on the market and no literature has been published regarding its efficiency. Thus, further studies will be required regarding this device.

- A further clinical study may be needed to corroborate the results of this study.

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APPENDIX

No	Material	Device	Surface	Control			BF			Wash		
				L*	a*	b*	L*	a*	b*	L*	a*	b*
1	PEEK	AQ	Polish	88.42	0.52	4.4	82.62	7.92	-4.09	86.94	3.88	1.72
2	PEEK	AQ	Polish	88.4	0.51	4.6	82.66	7.71	-3.95	87.03	3.53	2.05
3	PEEK	AQ	Polish	88.94	0.44	4.4	82.88	6.98	-3.46	87.08	3.62	1.9
4	PEEK	AQ	Polish	88.64	0.5	4.31	84.84	7.09	-3.04	87.97	1.47	4.01
5	PEEK	AQ	Polish	88.03	0.52	5.7	82.4	7.47	-3.42	86.83	2.74	3.9
6	PEEK	AQ	Polish	88.74	0.44	4.5	82.25	7.58	-3.44	88.15	2.44	3.08
7	PEEK	AQ	Polish	88.91	0.58	4.14	81.66	10.56	-3.58	88.6	1.69	3.59
8	PEEK	AQ	Polish	88.73	0.59	4.16	81.76	10.45	-1.65	88.44	1.53	4.24
9	PEEK	AQ	Polish	88.56	0.52	4.5	82.8	10.86	-2.88	88.5	1.55	4.3
10	PEEK	AQ	Un-polish	87.66	0.46	5.48	83.34	9.83	-2.02	87.66	1.96	3.09
11	PEEK	AQ	Un-polish	87.87	0.36	5.35	83.89	9.72	-1.75	87.5	2.79	2.76
12	PEEK	AQ	Un-polish	87.49	0.44	5.27	83.43	9.01	-1.04	87.44	2.02	2.95
13	PEEK	AQ	Un-polish	87.75	0.53	4.96	83.15	9.61	0.41	87.16	1	4.35
14	PEEK	AQ	Un-polish	87.97	0.52	4.7	83.72	9.71	-2.03	87.83	2.76	3.03
15	PEEK	AQ	Un-polish	87.72	0.51	5.4	84.48	8.96	-0.6	87.67	2.17	3.48
16	PEEK	AQ	Un-polish	87.62	0.47	5.41	84.25	12.83	-2.06	86.82	3.31	3.3
17	PEEK	AQ	Un-polish	87.44	0.6	5.89	83.86	12.19	-0.51	85.78	4.39	3.45
18	PEEK	AQ	Un-polish	88	0.51	5.23	83.8	12.09	-2.14	88.42	1.04	4.36
19	PEEK	WP	Un-polish	88.78	0.51	4.25						
20	PEEK	WP	Polish	88.59	0.55	4.36	82.55	7.03	-3.58	87.84	2.21	2.98
21	PEEK	WP	Polish	88.75	0.5	4.4	82.33	7.5	-3.37	87.58	2.27	2.65
22	PEEK	WP	Polish	88.93	0.43	4.38	82.38	7.45	-3.37	88.22	2.31	3.07
23	PEEK	WP	Polish	88.73	0.51	4.29	85.31	7.95	-3.73	88.76	1.23	4.02
24	PEEK	WP	Polish	88.28	0.53	4.65	83.75	7.45	-3.28	88.32	1.47	3.95

25	PEEK	WP	Polish	88.56	0.56	4.23	83.63	7.32	-3.59	88.43	1.66	3.5
26	PEEK	WP	Polish	88.27	0.51	4.56	83.84	10.76	-3.01	88.31	1.87	3.79
27	PEEK	WP	Polish	88.74	0.51	4.4	82.55	9.96	-5.03	88.33	1.38	4.28
28	PEEK	WP	Polish	88.85	0.61	4.33	82.48	10.13	-2.64	88.73	1	4.26
29	PEEK	WP	Un-polish	87.34	0.45	4.97	83.65	9.46	-1.14	87.73	2.51	3.93
30	PEEK	WP	Un-polish	87.83	0.51	5.07	83.35	9.27	-1.54	87.86	1.99	3.57
31	PEEK	WP	Un-polish	87.26	0.61	6.39	83.77	9.59	-1.06	87.27	2.57	3.45
32	PEEK	WP	Un-polish	88.26	0.49	4.89	83.53	9.03	-1.63	87.95	0.78	3.99
33	PEEK	WP	Un-polish	87.43	0.52	5.4	83.03	9.26	-2.28	87.52	1.63	4.18
34	PEEK	WP	Un-polish	88.04	0.51	4.61	84.25	9.24	-1.53	87.95	1.98	3.09
35	PEEK	WP	Un-polish	87.27	0.53	5.72	83.68	11.81	-2.43	87.06	2.62	3.8
36	PEEK	WP	Un-polish	87.97	0.51	5.2	83.76	12.5	-1.3	87.83	3	2.9
37	PEEK	WP	Un-polish	87.13	0.51	5.63	83.56	12.38	-1.42	87.08	1.41	4.46
38	PEEK	WP	Polish	88.52	0.63	4.42						
39	PEEK	WP	Polish	88.73	0.5	4.5						
40	PEEK	WP	Un-polish	87.49	0.56	5.4						
41	ZR	AQ	Polish	77.75	1.25	11.32	75.99	4.69	8.64	77.79	1.45	10.89
42	ZR	AQ	Polish	77.32	1.33	10.3	75.92	3.62	10.07	77.16	1.4	10.83
43	ZR	AQ	Polish	76.64	1.7	12.8	74.54	4.51	10.33	77.04	1.79	12.36
44	ZR	AQ	Polish	77.94	0.49	10.3	75.47	6.54	9.16	77.54	1.55	10.14
45	ZR	AQ	Polish	77.12	0.83	12.59	75.29	6.43	8.84	77.86	1.56	12.98
46	ZR	AQ	Polish	77.07	1.52	11.59	73.96	6.36	8.76	76.55	1.7	11.45
47	ZR	AQ	Polish	77.6	0.5	12.57	71.6	9.46	5.93	77.29	0.91	12.63
48	ZR	AQ	Polish	78.06	0.51	10.59	71.65	9.42	3.08	77.61	1.03	10.45
49	ZR	AQ	Polish	76.98	0.65	13.35	73.19	6.73	9.42	76.8	0.87	13.4
50	ZR	AQ	Un-polish	76.57	1.41	11.94	75.99	5.25	8.3	76.26	1.32	10.78
51	ZR	AQ	Un-polish	76.33	1.42	12.15	73.44	6.98	8.22	76.33	1.63	11.6
52	ZR	AQ	Un-polish	76.41	1.73	12.66	75.24	5.14	10.42	76.93	1.75	12.29
53	ZR	AQ	Un-polish	76.49	0.54	13.24	73.38	7.54	9.22	76.91	0.98	12.03
54	ZR	AQ	Un-polish	78.16	0.23	12.39	74.41	7.66	9.08	76.22	0.57	11.87

55	ZR	AQ	Un-polish	76.53	1.43	12.19	73.45	7.36	9.54	76.56	2.01	11.03
56	ZR	AQ	Un-polish	78.14	0.55	10.93	72.26	10.78	3.47	78.04	0.92	10.33
57	ZR	AQ	Un-polish	76.92	0.29	13.32	72.23	10.6	4.89	76.96	1.1	12.67
58	ZR	AQ	Un-polish	76.77	0.35	13.4	74.34	7.29	8.36	78.22	0.54	12.04
59	ZR	WP	Polish	76.91	1.38	11.1	75.33	3.96	9.88	77.49	1.34	10.75
60	ZR	WP	Polish	77.17	1.56	11.52	75.68	4.28	10.76	77.78	1.64	11.61
61	ZR	WP	Polish	77.3	1.4	11.01	75.32	4.2	9.9	77.16	1.53	11
62	ZR	WP	Polish	77.67	1.22	10.46	75.32	6.49	9.8	77.24	1.41	10.84
63	ZR	WP	Polish	77.07	1.68	12.18	75.67	6.18	9.58	77.3	2.07	11.85
64	ZR	WP	Polish	77.25	1.69	12.24	75.31	6.71	9.11	77.72	2.01	11.87
65	ZR	WP	Polish	78.05	1.03	10.43	74.05	6.73	9.67	78.08	1.07	10.71
66	ZR	WP	Polish	77.33	1.68	12.17	73.42	6.29	9.27	77.3	2	11.75
67	ZR	WP	Polish	77.03	1.52	11.73	73.42	6.47	9.4	77.12	1.76	11.62
68	ZR	WP	Un-polish	76.63	1.3	11.37	74.91	5.25	9.67	76.65	1.6	11.39
69	ZR	WP	Un-polish	76.77	1.46	12.89	76.63	5.53	9.33	76.96	1.54	12.44
70	ZR	WP	Un-polish	76.32	1.46	12.1	74.86	5.31	9.59	76.57	1.42	12.17
71	ZR	WP	Un-polish	76.28	1.5	11.93	73.11	7.51	8.51	76.47	1.44	11.65
72	ZR	WP	Un-polish	76.85	1.59	12.73	74.54	7.65	8.97	76.79	1.57	11.96
73	ZR	WP	Un-polish	76.66	1.76	12.68	74.57	7.12	9.36	77.01	1.73	12.12
74	ZR	WP	Un-polish	76.66	1.37	11.9	73.2	7.62	8	76.63	1.55	11.97
75	ZR	WP	Un-polish	76.96	1.63	12.22	74.35	7.4	9.37	77.36	1.52	12.08
76	ZR	WP	Un-polish	76.05	1.26	12.04	74.65	7.66	8.42	76.37	1.22	11.43
77	ZR	WP	Polish	78.18	0.27	11.84						
78	ZR	WP	Polish	77.08	0.82	12.76						
79	ZR	WP	Un-polish	76.9	0.8	13.35						
80	ZR	WP	Un-polish	77.06	0.37	13						
81	LDS	AQ	Polish	71.41	3.71	18.79	68.47	6.31	13.78	71.62	4.06	18.49
82	LDS	AQ	Polish	71.03	3.77	18.63	67.84	6.43	13.87	71.03	4.03	18.44
83	LDS	AQ	Polish	71.36	3.88	18.91	69.83	6.14	16.78	71.01	3.96	18.59
84	LDS	AQ	Polish	71.32	4.05	19.02	67.74	9.69	14.4	70.66	5.67	17.96

85	LDS	AQ	Polish	71.23	3.77	18.77	67.82	9.36	14.09	70.05	5.62	18.07
86	LDS	AQ	Polish	71	3.66	18.53	67.46	9.74	14.07	70.16	5.28	17.6
87	LDS	AQ	Polish	71.28	3.58	18.51	67.87	9.03	14.78	71.13	4.24	18.58
88	LDS	AQ	Polish	71.27	3.83	18.87	67.54	9.82	14.78	71.3	4.22	18.95
89	LDS	AQ	Polish	70.63	3.72	18.71	68.13	9.43	14.46	70.62	4.48	18.88
90	LDS	AQ	Un-polish	70.79	3.92	18.91	68.94	7.27	16.01	70.77	3.72	18.93
91	LDS	AQ	Un-polish	70.67	3.75	18.67	69	7.1	16.05	70.2	3.94	18.74
92	LDS	AQ	Un-polish	70.18	3.82	18.74	69.39	6.95	16.18	70.94	3.96	18.59
93	LDS	AQ	Un-polish	70.99	3.73	18.47	68.63	10.31	15.51	70.53	4.76	18.83
94	LDS	AQ	Un-polish	70.91	3.7	18.49	69.81	10.4	15.19	70.07	5.13	17.95
95	LDS	AQ	Un-polish	70.89	3.6	18.06	68.8	10.33	15.5	70.69	4.77	18.42
96	LDS	AQ	Un-polish	70.98	3.9	18.85	68.57	10.04	15.47	70.79	4.14	18.97
97	LDS	AQ	Un-polish	70.27	3.75	18.61	68.26	10.23	15.38	70.68	4.75	18.27
98	LDS	AQ	Un-polish	70.83	3.86	18.71	68.25	10.74	15.72	70.84	4.11	19.08
99	LDS	WP	Polish	71.57	3.71	18.5	70.19	6.59	16.56	71.48	4.42	18.33
100	LDS	WP	Polish	72.07	3.99	19.03	70.51	6.64	16.41	71.81	4.32	18.61
101	LDS	WP	Polish	71.53	3.86	18.92	69.52	6.83	15.58	71.29	4.29	18.5
102	LDS	WP	Polish	71.2	3.83	18.67	67.38	9.82	14.48	71.07	4.86	18.36
103	LDS	WP	Polish	71.32	3.96	18.84	68.55	9.45	14.52	70.99	4.62	18.13
104	LDS	WP	Polish	71.31	3.68	18.4	68.87	9.4	14.85	71.23	5.2	18.22
105	LDS	WP	Polish	71.5	3.69	18.65	67.66	9.71	14.37	70.84	3.96	18.37
106	LDS	WP	Polish	71.82	3.91	18.91	67.38	9.24	13.32	71.74	4.37	18.84
107	LDS	WP	Polish	71.42	3.45	18.28	67.2	9.58	12.73	71.39	4.09	18.76
108	LDS	WP	Un-polish	70.79	3.91	18.75	70.39	7.26	17.08	70.77	3.96	18.03
109	LDS	WP	Un-polish	71.96	3.97	19	69.63	7.04	16.53	70.24	4.01	19.22
110	LDS	WP	Un-polish	70.54	3.86	18.71	69.57	7	16.38	71	3.94	18.87
111	LDS	WP	Un-polish	71.28	3.92	18.69	68.51	10.29	15.34	70.66	4.22	18.84
112	LDS	WP	Un-polish	70.91	3.91	18.7	69.13	10.47	15.65	70.66	4.12	18.66
113	LDS	WP	Un-polish	71.79	3.89	18.67	68.4	10.81	15.62	70.64	4.67	18.74
114	LDS	WP	Un-polish	70.9	3.73	18.68	67.85	10.42	15.44	70.51	4.63	19.03

115	LDS	WP	Un-polish	70.91	3.7	18.5	68.95	10.83	15.04	70.43	4.16	18.82
116	LDS	WP	Un-polish	70.41	3.76	18.58	68.61	10.74	15.48	71.03	4.66	19.22
117	LDS	WP	Polish	71.93	3.73	18.71						
118	LDS	WP	Polish	71.41	3.83	18.63						
119	LDS	WP	Un-polish	71	3.61	18.5						
120	LDS	WP	Un-polish	70.39	3.8	18.7