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SURFACE ROUGHNESS – CAUSAL FACTORS – AND ITS RELATION TO BACTERIAL ADHESION

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To my whole dear family !

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And then.....?

ABSTRACT

Inflammation around teeth and dental implants is considered to be due to microorganisms producing biofilm and thereby initiating the inflammatory reaction. The etiology is not yet fully understood though many risk factors have been identified, e.g. smoking, oral hygiene, stress etc.

That surface roughness plays a role both in the development of the biofilm and discoloration of teeth is nowadays beyond doubt. To create a smooth surface is an important part of the oral hygiene regime. Toothbrushing with and without toothpastes can influence the surface roughness in different ways. The ultimate goal is to remove the biofilm together with the discoloration without removing the tooth substance and in the same time create a surface that is smooth in order to prevent the development of new biofilm and discoloration.

Hence the importance of having a reliable method for measuring toothpaste and toothbrush abrasivity is obvious. This is also applicable regarding the development of new dental filling materials especially in the anterior region where, besides bacterial accumulation, discoloration otherwise can be a problem.

The present thesis is based on four in vitro studies which all utilizes the profilometer technique for analysis of surface roughness caused by toothbrushing with and without toothpastes. In one of the studies, the additional effect of surface roughness on bacterial accumulation is investigated.

The main findings from the present thesis are that the profilometer technique, described in paper I, constitutes a possibility to measure the abrasive effect of a toothpaste or toothbrush in both a qualitative,(i.e. the roughness of the surface) and quantitative ,(i.e. how much of the surface that have been abraded), way. Furthermore, it has been shown that a softer toothbrush can cause equal or even more abrasion than a harder one and that toothbrushing with toothpastes on dental materials influences the materials in different ways, i.e. causing either rougher or smoother surfaces. Finally, the bacterial accumulation on titanium was not influenced by the surface roughness.

Although, these studies being in vitro studies, the impact on the clinical reality is huge. The “gold standard “ for measuring toothpaste abrasivity has been challenged, the opinion that softer toothbrushes always should be recommended is also questioned. It is extremely important for manufacturers of dental materials to consider the wear resistance as far as toothbrushing with toothpastes is concerned. Periimplantitis is a worldwide growing problem, where the present study has focused on surface roughness which constitutes an important aspect that needs further research.

Keywords: Abrasivity, toothpastes,toothbrushes, dental materials, biofilm

LIST OF PUBLICATIONS

This thesis is based upon the following papers, which will be referred to in the text by their Roman numerals:

I Liljeborg, A., Tellefsen, G., Johannsen, G. 2010. The use of a profilometer for both quantitative and qualitative measurements of toothpaste abrasivity. Int J Dent Hygiene 8, 237–243.

II Tellefsen, G., Liljeborg, A., Johannsen, A., Johannsen, G. 2011. The role of the toothbrush in the abrasion process. Int J Dent Hygiene 9, 284–290.

III Tellefsen, G.,Liljeborg, A., Johannsen, G., 2013. How do dental materials react on toothbrushing? Submitted.

IV Tellefsen, G., Linder, L., Liljeborg, A., Johannsen, G. 2013. The influence of surface roughness of titanium on bacterial accumulation / adhesion, in vitro. Submitted.

CONTENTS

LIST OF ABBREVIATIONS

BHI medium	Brain heart infusion
bis-GMA	Modified methacrylate
CHX	Chlorhexidine
DNA	Deoxyribonucleic acid
fimA	fimbrillin gene (a bacterial virulence gene)
FMC medium	growth medium specific for *S. mutans*
KHN	Knoop Hardness Number - a diamond indenter used in test
KTH	Royal School of Technology
M	mol/L (substance concentration SI unit)
Mil	a thousandth of an inch
N	Newton SI unit of force
P value	Statistical significance
PBS	Phosphate buffered saline
PCR-method	pellicle cleaning ratio
PCR technique	Polymerase chain reaction - a DNA sequencing technique
RDA	Radioactive dentin abrasion
REA	Radioactive enamel abrasion
R_a	Roughness value
R2	Symbol for the coefficient of determination of a linear regression
RNA	ribonucleic acid
SD	Standard deviation
SSCP	Diagnostic tool in molecular biology
S mutans IB	*Streptococcus mutans* Ingbritt (a serotype c strain)
TEGDMA	viscosity reducing agent
Å	Ångstrom, 10^{-10} m
nm	nanometer, 10^{-9} m
μm	micrometre, 10^{-6} m

INTRODUCTION

How it all started…….

Loma Linda, USA, year 1993 – everything in dentistry focused on periodontal regenerative procedures, dental implants and on cosmetics (Selvig et al., 1992; Israelson and Plemons, 1993). The plaque theory was not controversial anymore. What was seen was a very progressive and positive attitude in dentistry. Dental companies only saw unlimited possibilities, opportunities were countless and unrestricted. Clinicians were very cherished by these companies. The Swedish state insurance company "Försäkringskassan" approved treatment without any deeper discussions. The cost-benefit aspect was not on the agenda.

Department of Dental Medicine, Huddinge, year 2008 – a growing sense of and facts arose that complications and failures more and more often were seen. Not always immediately, sometimes after some years and over decades (van de Velde et al., 2009). How predictable were periodontal regenerative procedures? What to do with peri-implantitis? The concept "peri-implantitis" was not so well known back in 1993.

Restorations, i.e. crowns and veneers, especially those made from an esthetical standpoint only, had their advantages but also disadvantages. Margins and edge connectivity of the restorations created problems to maintain the gum line. The euphoria began to subside and we saw more of classical, conservative ways to save teeth like e.g. apically repositioned flap procedures to maintain molars. The need for dental hygiene became more and more obvious. Dental hygienists have an established position both in general, as in more advanced dentistry in Sweden today. By saying this, the circle is complete.

Oral hygiene, plaque control, pocket measurements, overhangs, roughness, and minimizing bacterial load is still of utmost importance to prevent disease and to maintain what we have achieved.

The present thesis focuses on the relationship between surface roughness, caused by toothbrushing with and without toothpastes, and bacterial accumulation.

To be able to understand the full scope and importance of this research it must be kept in mind that the turnover for toothbrushes and toothpastes in Sweden well exceeds one billion SEK every year. The market is overwhelmed with different toothpastes and toothbrushes, all claiming different effects, e.g. against sensitive teeth, anti-plaque, gingivitis, bad breath and claiming staining removal ability. The extensive use of mouth-washes and rinses, e.g. chlorhexidin (CHX) causing stains have also created a need for toothpastes with effective stain-removal properties and low abrasivity. Furthermore CHX can also enhance staining from sources like tobacco use and the diet. (Koertge, 1997). According to the American dental

association health foundation 1984 there is a linear correlation between dentin abrasivity and stain removal in a study where calcium ortho-phosphates was used as an abrasive agent.

Later in-vitro studies, though, has shown new toothpastes formulas both with a low abrasivity and with stain removal performance comparable with that of benchmark marketed pastes (Creeth et al., 2006).

Historically, tooth cleaning agents with abrasive contents dates back over 2000 years, e.g. powdered marble, seashells, powdered coral, all aiming at the same goal - "whiter teeth".

Needless to say, it is important to understand that the whitening effect is only defined in the sense of ability of taking away discoloration and deposits from the tooth surface more effectively and not by creating a whiter surface itself. Not to be confused with bleaching which can lead to whiter teeth (chemically), which on the other hand has showed to cause surface roughness and adhesion of *S.mutans* to enamel (Hosoya et al., 2003).

In light of this it is important not only to have a reliable method for measuring the abrasivity of a toothpaste, but also to understand the influence of the toothbrush in the abrasion process, as well as the interaction between the effect of the toothbrush and the toothpaste. Mostly, so called soft toothbrushes are recommended today. There is a well established opinion that, so called harder toothbrushes always cause more abrasion. Nygaard-Ostby, (1979) focused on the poor analysis previous done, and how the brushes were classified, e.g. stiffness. He recommended a standard regarding how to categorize toothbrushes. The soft and medium toothbrushes are today well-defined.

The knowledge concerning abrasion has to be applied when developing new dental materials especially for use in the anterior region since a rougher surface more easily attracts plaque (biofilm) and causes discoloration (Quirynen et al., 1995; Takayoshi, 2002; Hosoya et al., 2003). Finally, the significance of these investigations has to be understood with regards to the effect of surface roughness on bacterial accumulation. The findings from the present thesis address all these important issues.

Abrasion

For the majority of us, the appearance of the teeth is very important, and any discoloration or stain that may form on them will affect their esthetic qualities. Subsequently, abrasion on teeth and dental materials is an important topic and have throughout the years been associated with toothbrushing with toothpastes. (Stookey et al., 1982; Barbakow et al., 1987; Joiner et al., 2006; 2008; Voronets

et al., 2010). The term abrasion must not be confused with attrition and erosion: attrition being the loss of tooth structure by mechanical forces from opposing teeth and erosion being the irreversible loss of tooth structure due to chemical dissolution by acids not of bacterial origin.

It has been discussed whether a toothpaste with a high abrasive value (radioactive dentin abrasivity-RDA) is more harmful to the teeth than a toothpaste with a low value (Hefferren et al., 1976; Vincentini et al., 2007; Joiner et al., 2008).

The abrasive effect of toothbrushes, or the contributing effect of a hard or soft toothbrush, to the abrasive effect of toothpastes has also been in focus for research (Dyer et al., 2000). Enamel has a hardness (KHN – Knoop hardness number) value of 320, dentin between 50-60, and most abrasives used in toothpastes of today have hardness values somewhere between 50-150, thus making wear of enamel due to toothpastes a non- existing problem. This would only be a problem if the abrasives used were harder than enamel. Joiner et al., (2002) visualized this in a study comparing a whitening toothpaste and a standard silica toothpaste. They showed that the whitening toothpaste was more effective in stain removal, however when extrapolating the results to show if there would be a risk for harmful wear, they found that it was not possible to wear away any significant amount of enamel even after a lifelong brushing with a whitening toothpaste.

When stating that enamel is not harmfully influenced by toothbrushing with toothpastes, it must be kept in mind that areas that are not covered with enamel e.g. in patients with exposed tooth necks or patients treated for severe periodontitis, dentifrice abrasivity can constitute a problem. Since the hardness of most abrasive agents in toothpastes exceeds that of human dentin, the risk for dentin abrasivity must be taken into consideration. However, the scientific results in that matter are inconclusive, and interestingly some authors did not find any association between abrasion and oral hygiene factors (Bergström and Eliasson, 1988; Sangnes and Gjermo 1976; Volpe et al., 1975). Zimmer et al., (2005). Dentin abrasion in vitro was evaluated following professional tooth cleaning, using a profilometer technique. They compared prophy brushes and prophy cups with four different abrasives each (calcium pyrophosphate, pumice, Hawa cleanic and Nupra course) giving a total of eight different tooth cleaning procedures on dentin specimen. No statistical differences between brushes and cups were found and they also concluded that none of the procedures represented any major risk for dentin loss.

It has been widely accepted in the dental profession that some degree of abrasivity is needed in a toothpaste if satisfactory cleaning of the teeth is to be achieved (Stookey et al,1982; Forward, 1991). On the contrary, recently, no contributing effect to the mechanical plaque removal by the use of toothpaste was

found (Paraskjevas et al., 2006) however no aspects of stain removal were taken into consideration.

To measure the abrasive effect of toothpastes many different techniques have been used over the years (Hefferren, 1976; Redmalm and Rydén, 1979). Both quantitative and qualitative techniques have been used. Gravimetric measurements (Franz, 1974; Hotz, 1983) and radio tracer techniques are examples of quantitative techniques where the amount of substance removed is analysed, whereas light reflexion techniques (Murray, 1971; Elmer et al., 1975; Redmalm and Rydén, 1979) and surface profile measurements (Franz, 1977; Kielbasa et al., 2005) have been used as qualitative techniques, to evaluate the appearance of the surface after brushing

VARIOUS TECHNIQUES that have been used include:

Weight loss technique: the test samples are weighted before and after the abrasion procedure. The weight loss represents the measure of the wear (Cornell et al., 1957; Shell et al., 1966; Kanter et al., 1982; Harrison et al., 1985).

Loss of thickness: may be calculated by using a formula:

$$\text{Thickness loss} = \frac{\text{weight loss x original weight}}{\text{original weight}}$$

(Harrington et al 1982, Heath et al 1983, Jones et al 1985)

Volume loss techniques: It usually means the use of pycnometry-a method used to determine the density of a liquid-and then dividing the density of the sample with the weight loss (Mahalick et al 1971, Aker 1982, Staffanou etal 1985, Schulte et al 1987).

Radiotracer technique: To date the most commonly used and accepted way to describe the abrasivity of a toothpaste is through the use of a radio tracer technique, the radioactive dentin abrasivity (RDA)-value. This is a quantitative technique based on irradiation with neutrons of the tooth substance in the test material which converts phosphorous of the hydroxy apatite of the dentin to its radioactive isotope. After brushing with a toothpaste, the substance abraded from the surface of the specimen is measured with a Geiger counter. The amount of substance removed is calculated and compared with a reference paste (Grabenstetter et al., 1958; Hefferren et al, 1976). The reference paste is usually calcium pyrophosphate and the abrasivity value is set to 100 and the

abrasivity of the test paste is expressed in relation to this value. The RDA-value gives an estimate of how much of the surface that has been abraded, that is, a quantitative measurement of surface abrasivity. It does not measure the roughness of the abraded surface. The description above is also valid for abrasivity values regarding enamel – REA (radioactive enamel abrasivity). Barbakow et al., (1987) compared the radiotracer method with the gravimetric method and found only four brands of toothpastes out of 21 were equally ranked. They saw drawbacks for both methods, e.g. morphological changes of brushed dentine were difficult to standardize. Surface dentine has fewer tubules per unit compared to deeper specimens.

Surface profile measurements: In order to further evaluate the abrasivity of a toothpaste, the quantitative measurement should be completed with a qualitative measurement, for example, by using a profilometer. Profilometer techniques have been used in earlier studies (Dyer et al., 2000; 2001; Addy et al., 2002; Kielbassa et al., 2005). In a study by Davis and Winter, (1976) a profilometer was used to evaluate the abrasivity of different toothpastes on human enamel. They measured the mean depth of the profiles and presented the results as a percentage of the abrasion of chalk which was set to 100. Modern profilometers like the one used in the present thesis express the surface roughness in µm, using the Ra – value which is a mean arithmetic value of the surface roughness. As the roughness is not only strongly correlated to bacterial accumulation, but also to the 'lustre' of the tooth (Redmalm et al., 1981; 1985), such measures are important and have a great clinical relevance. Another surface profile measurement technique is scanning electron microscopy (SEM) . By combining the SEM with a digitizier connected to a computer the mean arithmetic roughness value can be calculated (R_a) (Leitao, 1984).

Light reflexion measurements: this technique is based on the registration of light reflected from a surface. The principle of measuring surface roughness with a light source is old (Schmaltz 1936). Murray, (1971) showed that laser as a light source offered some advantages as compared to ordinary light. Redmalm and Rydén, (1979) developed a laser reflexion apparatus to be used for laboratory studies of dentifrice abrasivity. The investigators showed 1987 that it was possible to apply the laser reflexion technique for in vivo investigations of surface changes following toothbrushing. Johannsen et al., (1993) compared the cleaning effect of toothbrushing with three different toothpastes and water using the laser reflection technique.

Test specimen

Abrasion studies have been performed in vitro using various specimens of enamel (hydroxyapatite) and dentine. Acrylic plates with the same hardness as dentine have also been used and been shown to be appropriate for comparative studies of dentifrice abrasivity (Addy et al., 1991, Dyer et al., 2001). The reproducibility is high for acrylic plates. One study compared human and bovine dentine specimens and no significant difference between them was recorded. Wegehaup et al., (2010). Fonseca et al., (2008) found that bovine dentin hardness (measured in KHN) differs with the age of the animal and that it was a discrepancy in hardness when compared with human dentin. They recommended as a general rule to use older bovine teeth due to better chances to find greater similarity with human teeth. Drawbacks such as morphological changes of brushed dentine and that surface dentine has fewer tubules per unit were discussed by Barbakow et al., (1987).

The purpose of the study was to compare the abrasivity of different toothpastes and to correlate these relative results with other methods. There was no intention of making absolute measurements of the abrasivity and therefore we found it satisfactory to use these acrylic plates. And again similar specimens have been used in other studies (Dyer et al., 2001).

The development of novel composite filling materials started when methyl-methacrylate was introduced into dentistry during the 1930s, which in the beginning was a denture –based material hardened by heat curing. During the 1940s researchers were able to cure methacrylates by a cold curing process, thus making it possible to use in the oral cavity. To reduce the problem of shrinkage, dimethylmethacrylate, i.e. bis-GMA (Bowen's resin) was created. Bowen's resin is an important ingredient in composite fillings of today.

In recent years dental filling materials containing amalgam have been replaced by composite materials, which are now being used in all areas of the mouth. The composites used in the anterior region often contain bis-GMA with filler particles 30-60% by weight, while in the molar region the amount of filler particles can reach 83% using hybrid composites. By using three different particle sizes the filler load can be as high as 90 %. The composites have during the years been improved to withstand chewing forces in the molar region. They have also been modified either to be used in the anterior or the posterior (molar) region of the mouth. It is of utmost importance that these materials are not influenced negatively by tooth brushing with toothpastes or water, since increased surface roughness will lead to discoloration and plaque accumulation, which would consequently lead to increased risk for caries and gingivitis (Quirynen et al., 1995; Hosoya et al., 2003).

Biofilm formation

Antonie van Leeuwenhoek (1684) was the first to display the "animalcules" (bacteria) found in plaque scraped from his teeth, and described in a report to the Royal Society of London: "The numbers of these animalcules in the scurf of a man's teeth are so many that I believe they exceed the number of men in a kingdom." In the 1940s Heukelekian and Heller in an issue of the Journal of Bacteriology wrote that development takes place either as bacterial slime or colonial growth attached to surfaces, and Zobell (1943) noted an "effect" in seawater and described many of the fundamental characteristics of attached microbial communities (Paraje, 2011). Biofilm formation on oral surfaces plays an important role in oral infections. Bacteria that adhere to oral surfaces aggregate in a bacterial polymeric matrix to form biofilms. Examples of biofilm induced infections are dental caries, periodontitis and peri-implantitis.

Biofilms can grow on all parts of the body, as well as on artificial devices e.g. catheter, pace-makers etc. Regardless of the location they share several common features e.g. the synthesis of an extracellular matrix that holds the bacterial cells together, thus increasing the resistance to be destroyed by the host defence (Mah and O´Toole, 2001). The mechanism by which the matrix contributes to antimicrobial resistance is either by acting as diffusion barriers or by binding directly to antimicrobial agents. The development of the biofilm is categorized into three stages. First stage being the attachment to a surface and growth into a sessile colony, second stage involves the multiplication of the bacteria and synthesis of an extra cellular polymeric matrix, thus leading to pillar and mushroom shaped masses which also contain fluid filled channels serving as a primitive circulatory system, and using quorum-sensing to communicate (Hall-Stoodley et al., 2004). The final stage comprises the detachment of cells from the biofilm colony and their dispersal into the environment and disease transmission.

It has been claimed (National Institutes of Health) that more than 60% of all microbial infections are caused by biofilms. Bacterial exopolysaccharides are important as extracellular matrix of the biofilm and contribute to bacterial adhesion and coaggregation and help biofilms to grow. Mutans streptococci are well-known producers of both water insoluble and water soluble exopolysaccharides from sucrose. (Costerton et al., 1999; Mah et al., 2001; Wilson, 2005; Pamp et al., 2009).

Periodontal diseases

is initiated by the accumulation of microbial plaque above the gingival margin, which extends into the subgingival environment. Dental plaque consists of a biofilm that induces an inflammatory response in the tissues, leading to increased leakage of fluid from the small vessels (capillaries) and movement of acute

inflammatory cells (neutrophils) into the tissues and into the gingival sulcus. This leads to cellular and morphological changes in the connective tissue and some of the collagen in the connective tissue is lost. Eventually, immune cells (lymphocytes) and neutrophils start to accumulate in the area below the sulcular epithelium. This stage is defined as gingivitis and has the classical signs of inflammation, including redness, swelling and pain when the tissue is probed.

The mechanisms behind the shift from gingivitis to periodontitis have not been clarified, however it has been shown that bacteria are needed to create tissue destruction. A dramatic increase in the number of neutrophils and chronic inflammatory cells (macrophages) is one of the changes that occur. There are evidence pointing to the fact that the transition from gingivitis to periodontitis is triggered by dysregulation of the host response, which leads to an exaggerated inflammatory response. (Terheyden et al., 2013; Janssen et al., 2013)

Peri-implant diseases

Infections affecting dental implants, e.g. peri-implant mucositis and peri-implantitis is a worldwide growing problem. The prevalence at the individual level for peri implant mucositis is ranging from 48-80 %, and for peri-implantitis 15-56 % (Roos-Janåker et al., 2006; Lindhe and Meyle, 2008; Zitzmann and Berglundh, 2008; Atieh et al., 2012). This represents a health-economic problem both on an individual level as well as for the society at large. The impact of surface properties of implant materials on bacterial adhesion and accumulation has also been investigated (Quirynen et al., 1993; 1996; Bollen et al., 1996; Bürgers et al., 2010).

Patients at risk for peri-implantitis are patients with periodontitis. (Simonis et al., 2010). But at least mucositis and gingivitis studies with broad-range PCR techniques have shown that, the microbial diversity of the investigated implants and teeth with clinical signs of mucositis or gingivitis exhibits substantial differences demonstrating that, transmission of the complete bacterial microflora from teeth to implants could be excluded (Heuer et al., 2012). Full mouth extraction does not result in eradication of all periopathogens but only in significant reduction. Nine patients with severe, aggressive periodontitis were followed. 6 months after extraction a 3-log reduction of *P. gingivalis* and *T. forsythia* and a more modest reduction of *A. actinomycetemcomitans* and *P. intermedia*, however detection in saliva and on the tongue remained unchanged (van Assche et al., 2009).

Biological factors contributing to failures after periodontal treatment as well as after implant treatment have been discussed such as medical status of the patients, smoking, bone quality, implant surface characteristics and design (Preber and Bergström, 1990). In early implant losses excessive surgical trauma and impaired

healing ability, premature loading and infection are important factors. Late failures can be explained by such factors as progressive chronic marginal infection (peri-implantitis), overload and host characteristics.

A factor that must be taken into account when discussing abrasion of titanium is the pH –value of the water- toothpaste slurry (Hossain et al., 2006). They suggested that a smoother surface can be obtained using more acidic slurries compared to the neutral ones, by releasing titanium ions from the surface.

Busalmen (2001) showed that with increased ionic strength (0.1M and 0.6 M) and also by modifying the pH (2 to 8) the adherence of Pseudomonas species to titanium changed. Maximum bacterial adhesion was obtained with a 0.1 M of the electrolyte solution at pH 6. At 0.6M solution an absence of bacterial adhesion was observed throughout the pH range tested. They concluded that changes in adhesion had to do with changes in the number of reinforced H-bond forming sites on the titanium surface.

Surface energy also plays a role. Absolutely pure titanium surfaces exhibit high surface energy, high hydrophilicity, which is important for integration and osteoblast differentiation. By adsorbtion of hydrocarbons and carbonates from the ambient atmosphere, surface energy and hydrophilicity decreases . By novel hydroxylated/hydrated titanium surfaces this high surface energy of TiO_2 can be retained (Zhao et al, 2005).

Further increasing hydrophilicity of the biomaterial surface might be beneficial for minimizing the biofilm. By different coatings titanium hydrophilicity can be increased and reduction in biofilm formation is possible (Gasik et al., 2012).

Mabboux et al., (2004) showed that *S. sanguinis* with hydrophobic properties and *S.constellatus* with hydrophilic properties adhered differently to titanium. *S. sanguinis* adhered in higher numbers than *S. constellatus*. Saliva coating can in some reports increase the biofilm formation. Using saliva as a culture medium could be adequate to simulate an in vivo situation when performing adhesion studies. However, Almauger et al., (2012) claimed that saliva both can stimulate and inhibit bacterial growth.

It is possible to maintain the hydrophilicity by preventing the implants from air exposure and keep them stored in isotonic NaCl solution. With a contact angle of 0º, this represents an extremely hydrophilic surface. These surfaces are osteogenic. Usually titanium surfaces tend to have low surface energy and thereby are hydrophobic.

The surfaces with an increased osteoblast proliferation exhibited particularly higher surface roughness (R_a - 3.5 μm) followed by a high polar part of the surface free energy whereas the effect of wet ability played a minor role. (Kubies et al., 2011). Others pointed out the relevance of wetability and osteoblast proliferation (Park et al.,2011).

Roughness of different dental materials in relation to bacterial accumulation and growth were in focus already twenty years ago. Leonhardt et al., (1995) investigated titanium, hydroxyapatite and amalgam and found no significant differences between the materials regarding colonization of the bacteria investigated. However, conflicting data have been presented. Busscher et al., (2010) claimed, that more biofilm accumulated on rough than on smooth surfaces. In contrast, Barbour et al., (2007) found that titanium polishing did not reduce oral bacterial colonization. Likewise, Größner et al., (2001) showed that no differences were seen between polished and laser treated titanium (which is rougher) in terms of bacterial colonies.

Surface properties such as roughness have an impact on failures (Esposito et al., 1998). It has been shown that the machined surfaces of implants showed higher incidence of early failures, but decreasing over time, while rougher surfaces lower incidence of early failures, but increased failure rate over time (Esposito et al., 2007; Charalampakis et al., 2012).

Experimental periodontitis and peri-implantitis animal models have shown that the surface properties affects the inflammatory process and the magnitude of the resulting tissue destruction. (Carcuac et al., 2012)

Surface roughness however, was not the issue in studies by Größner et al., (2001) and Elter et al., (2008) where they showed that the subgingival biofilm accumulation was not influenced by higher surface roughness in subgingival areas.

Contradictory to this at a consensus meeting it was stated; “Bacterial biofilm formation on implant surfaces does not differ from that on tooth surfaces, but may be influenced by surface roughness. There is no evidence that such differences may influence the development of peri-implantitis” (Lang and Berglundh, 2011).

AIMS

Overall aim:

The overall aim of the present thesis was to investigate the abrasion caused by toothbrushing with and without toothpastes on teeth and dental materials and the relation to bacterial accumulation. By using a unique combination of technical equipment and specimens the aim was also to challenge "the gold standard" RDA-value (radioactive dentin abrasivity), when measuring abrasivity.

Specific aims:

- To evaluate the abrasivity of different toothpastes both quantitatively and qualitatively with a profilometer technique and correlate these measurements to the RDA values.
- To evaluate the relative abrasivity of different toothbrushes both qualitatively and quantitatively.
- To investigate if and how, different filling-materials are affected by brushing with and without tooth pastes
- To evaluate the effect of titanium roughness and of the composition of the growth medium on biofilm formation.

PAPERS I-III

MATERIALS AND METHODS

BRUSHING MACHINE in studies I-III

The equipment has a reciprocating movement of 85 mm; 2000 double strokes per hour; and load of 2.35 N. It holds six brush sites, and each brush site had a trough for the toothpaste water slurry in which the test plates were placed. Between each test, new brushes were mounted in the machine.

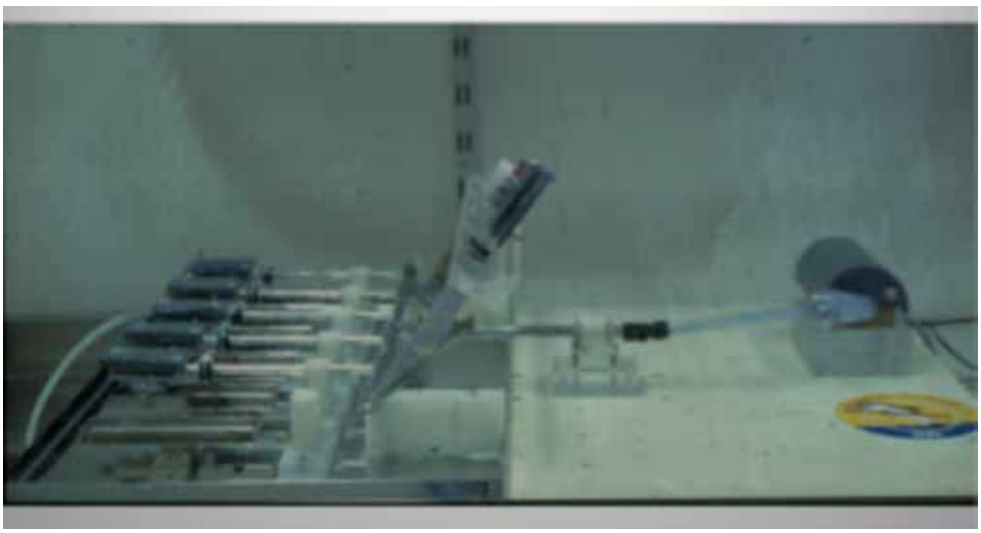


Figure 1: *Photograph of the brushing machine with the six brushing sites. One holder for the brush is upright.*

In study I and II - every hour the plates were removed and rinsed in lukewarm water, and the slurry was refilled. The total brushing time was 6 h corresponding to 12 000 double strokes, but the plates were also analyzed after 1 h brushing (2000 double strokes).

The reason for choosing 2000 and 12000 double strokes were our attempt to translate these results into a clinical reality. Many authors have extrapolated their results into corresponding brushing time in real life such as Sexson & Phillips (1951). See more under discussion.

In study I - the procedure was carried out with eleven different commercially available toothpastes. The abraded area was covering the full length of the acrylic plate (Fig. 1).

In study II - the procedure was repeated for all the ten brushes.

In study III - two plates of each material were brushed with one toothpaste, two with another toothpaste and two plates were brushed with only water.

The brushing machine was also tested on the titanium in study IV, but since we did not see any effect in terms of polishing nor scratches from toothbrushing we decided to stress the limits. By starting from the machined surface and polish to a highly glossy mirror shaped surface, and finally by using 250 µm aluminum oxide particles a very rough surface was also achieved.

PROFILOMETER IN ALL FOUR STUDIES

The roughness was evaluated in a Profilometer, measuring the R_a-value. R_a is defined as the arithmetic average deviation of the absolute values of the roughness profile from the mean line or the center line. The surface profilometer- P15 from company KLA Tencor Corp., San Jose, CA, USA has a diamond tip with a radius of 2µm. It has been used in all four studies, and has an ability to detect structure unevenness on a surface of about 50 nm. The ultimate lateral resolution is 25 nm in x-direction and 1 µm in y-direction. However, the actual resolution is a function of stylus radius. The scan repeatability is 7.5 Å or 0.1% of step height, and the reproducibility is 15 Å or 0.25% of step height (manufacturers specifications).

Figure 2: *The lab at KTH. Fume hoods.*

Figure 3: *Photograph of the profilometer on its gyro-stabilized table.*

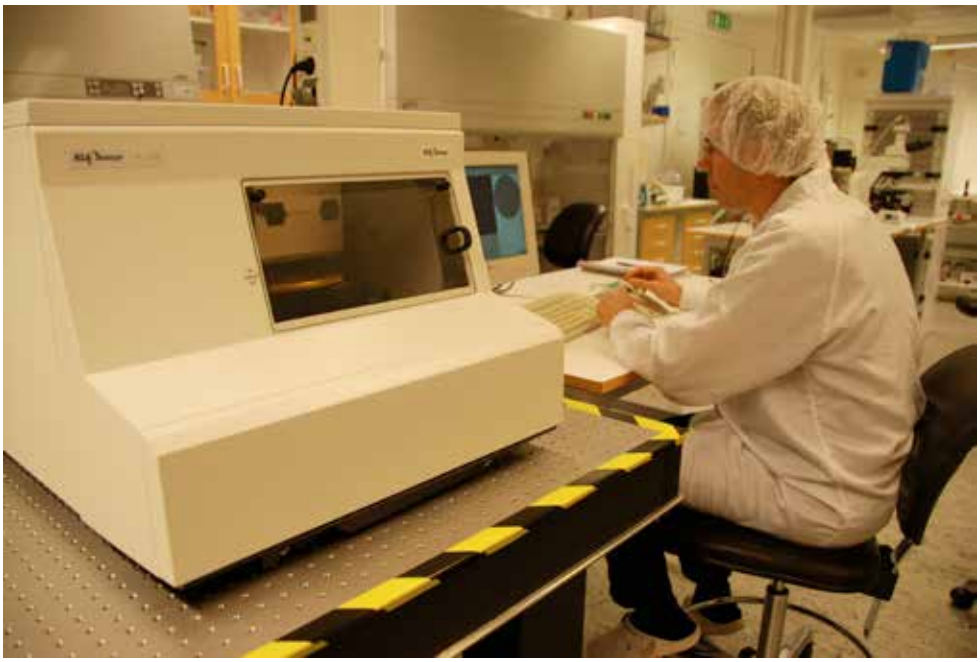


Figure 4: *The profilometer. Registration of the roughness values on the computer*

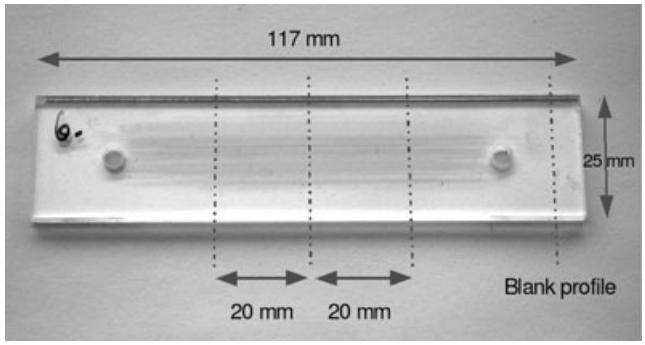


Figure 5: *Plexiglass specimen with indications where the profiles where collected*

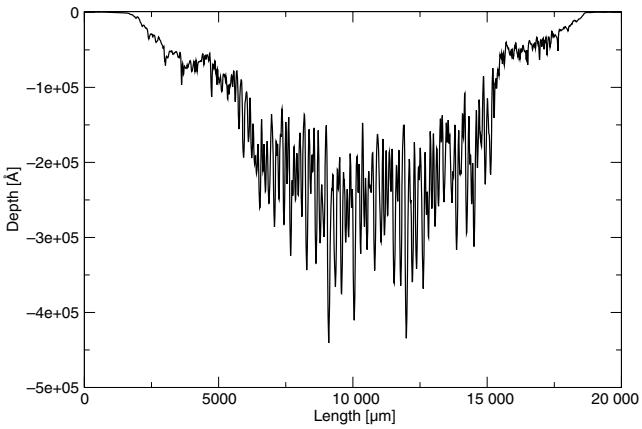


Figure 6*: Typical profile with straight ends*

Table 1: Distribution of the different methods used in the four studies.

STUDY	BRUSHING-MACHINE	PROFILOMETER	MICROBIOLOGY
I	X	X	
II	X	X	
III	X	X	
IV	(X)	X	X

ABRASION STUDIES PART (PAPERS I-III)

The three abrasion papers included the brushing machine in the same way but also the profilometer. Acrylic specimens were used in a similar way regarding paper I and II. In paper III the acrylic plates only served as holders for the dental material specimens. The outcome of the studies indicates that this unique combination of equipment can provide a platform for further investigations.

PAPER I

Toothpaste and acrylic specimen preparation:

Table 2: The following eleven commercially available toothpastes containing different abrasives were used (the manufacturers given RDA values are specified).

	ABRASIVES	PRODUCT NAME	RDA
A	Sodium metaphosphate	Acta Original®	40
B	Sodium metaphosphate	Acta Proactive®	40
C	Silicone dioxide	Colgate ´blå mintgel´®	70
D	Silica	Aquafresh for kids®	50
E	Silica	Sensodyne fresh sensitive®	55
F	Calcium phosphate, Calcium carbonate and Aluminium silicate	Clinomyn for smokers®	130
G	Silicone dioxide	Pepsodent Crystal Fresh®	79
H	Silica	Zendium Classic®	50
J	Silica	Bamse (for children)®	55
K	Silica	Zendium Dentin Sensitive®	30
L	Silica	Theramed Ice Fresh®	50

All brushes in this study were of the same brand (TePe vågig® soft). Three acrylic plates were mounted in the brushing machine and the toothpaste water slurries was added. The slurry contained 25 mg of toothpaste and 50 ml of water. The plates were then analyzed using the surface profilometer.

Laboratory:

Every hour the plates were removed and rinsed in lukewarm water, and the slurry was refilled. The total brushing time was 6 h corresponding to 12 000 double strokes, but the plates were also analysed after 1 h brushing (2000 double strokes). This procedure was then repeated for all the eleven toothpastes.

Study design:
Three profiles were collected for each sample (Fig.5), one at midpoint, and two profiles 20 mm above and below the midpoint. Profiles were also collected outside of the abraded area to measure the curvature of the clean sample surface. The profiles were used to compute the volume of removed material between the upper and lower profiles. Also the roughness average (R_a-values) were computed for the centre 20% length in each profile. The volume values and R_a-values were then correlated to each other and also to the RDA-value received from the manufacturer of the toothpaste using standard line fitting procedure.

Statistical analysis
SDs for R_a and volume loss were calculated. R_a versus volume loss, RDA values versus volume loss and RDA values versus R_a were computed. Student t-test were used to obtain the p-values. R^2 designates the square of the Pearsons correlation coefficient.

PAPER II

Toothbrush and acrylic specimen preparation:

Table 3: 10 commercially available toothbrushes were used.

TOOTHBRUSHES	NO. OF FILAMENTS	FILAMENT DIAMETER (mm)	FILAMENT LENGTH (mm)
TePe x-mjuk	2856	0.13	11.32
Dentosal	2720	0.12	10.62
Jordan soft	2052	0.12	11.20
TePe select, mjuk	1794	0.16	10.88
Pepsodent essential	1748	0.12	10.68
Oral B cross A	1680	0.14	11.10
Jordan medium	1330	0.14	10.84
Oral B, barn	896	0.14	9.09
Butler gum 431	884	0.19	11.05
TePe vågig, mjuk	816	0.20	11.40

Figure 7: *Examples of toothbrushes (Trimmed to fit the brushing machine).*

One toothpaste was used in this study and as control served water alone. Three plates were mounted in the brushing machine and the toothpaste water slurry was added. The slurry contained 25 mg toothpaste and 50 ml water. The plates were then analyzed using the surface profilometer.

Clinomyn for smokers®, contains the following abrasives; Calcium phosphate, Calcium carbonate and Aluminum silicate (From the manufacturer specified RDA value – 130)

Laboratory:
Every hour the plates were removed and rinsed in lukewarm water and the slurry was refilled. The total brushing time was 6 h corresponding to 12 000 double strokes, but the plates were also analyzed after 1 h brushing (2000 double strokes). This procedure was then repeated for all the ten brushes. The abraded area was covering the full length of the acrylic plates. The profilometer scanned the surface profile of the sample in a direction perpendicular to the brushing direction.

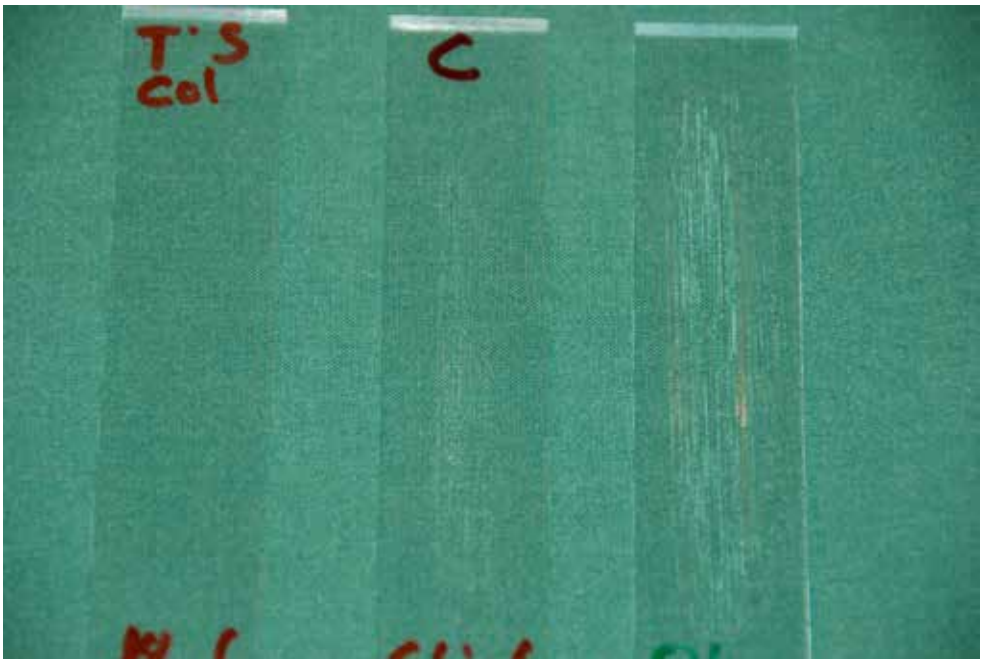


Figure 8: *Various brushing appearance after brushing 6h. To the left toothbrush and water. In the middle and to the right with Clinomyn®, with two different toothbrushes.*

Study design:
Three profiles were collected for each sample (Fig. 5), one at midpoint , and two profiles 20 mm above and below the midpoint. Profiles were also collected outside of the abraded area to measure the curvature of the clean sample surface. The profiles were used to compute the volume of removed material between the upper and lower profiles. Also the roughness average (R_a-values) were computed for the centre 20% length in each profile.

Statistical analysis:
The significance of the difference in the abrasion values between the toothbrushes was calculated using unpaired t-test (for calculating equality between means). The t-test was also applied on the abrasion values over time. Correlation between R_a and volume measurements and between number of filaments and abrasion values was calculated using Pearson's correlation test (SPSS 13.0, Statistical Package).

PAPER III

Filling materials tested were:

Tetric Ceram HB® (Heavy Body), Ivoclar. Capsules – Bis-GMA 19w%. Fillers 81w%. Particles sizes 0.04-3.0 um. Multi fractions.

Charisma® Heraeus. Capsules – Bis-GMA and TEGDMA (reducing viscosity). Fillers 78w% Two filler fractions 0.01-0.07 resp 0.7 – 2.0 um.

Dyract® Flow Dentsply. Syringe. A compomer – alkyle-, aryle or alkylearyle esters from monomethacrylateacid 25-50%.

Grandio® (Voco) capsules – Bis-GMA. (Modified methacrylate) 2.5 – 5 %. Fillers 87 w%.

TAB 2000® (Swedon), cold-cured acrylic – methacrylate. Methylmetacrylate more than 90 %.

The toothbrush used was TePe Straight Classic®. The toothbrushes were manufactured according to the ISO standard 20126:2005 where the properties are defined and the general requirements and test methods regarding physical inspection, tuft removal force, fatigue resistance and chemical challenge are described. Toothpastes were Pepsodent Whitening® and Colgate Smiles®.

Preparation of the dental material specimen:

The composite materials were cured in three different locations close to the borders and in the centre for 2 x 20 second on acrylic plates. The light curing process was then repeated on the other side of the plate. This is considered satisfactory according to Caughman et al., (1995). Curing light unit used was "Demi LED, 921640" from Kerr®. The TAB 2000 is a self and cold cured material. The curing was confined between two acrylic plates; the prepared plate, mentioned above and one untouched on top, resulting in a comparable surface structure. The two plates were fixed together with two clamps for at least ten minutes using a force of approximately 40 N. The plates were then subjected to brushing in the brushing machine with a toothpaste-water slurry (25 mg toothpaste + 50ml water). The slurry was renewed every hour and the two different toothpastes were used, separately.

The total brushing time was six hours corresponding to 12000 double strokes but the plates were also analyzed after one hour of brushing (2000 double strokes). All together sixty plates were manufactured twelve of each material. Two plates of each material were brushed with Colgate Smiles, two with Pepsodent Whitening and two plates were brushed with only water.

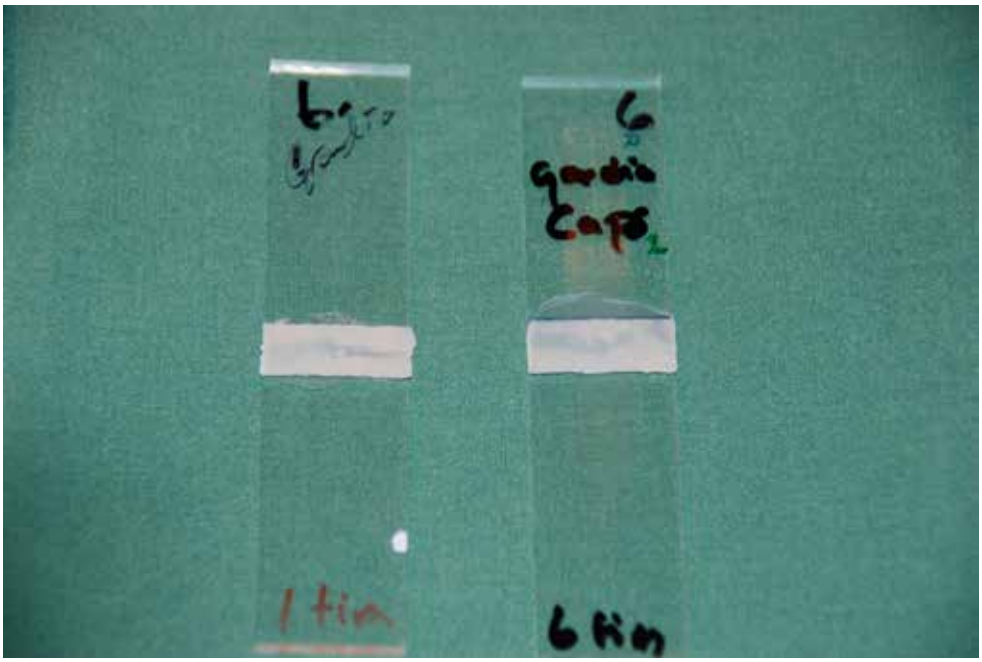


Figure 9: *Filling material specimens with different degree of roughness after brushing in the brushing machine*

Study design:
Three profiles were collected for each sample, one at midpoint of the plate and two profiles 3 mm above and 3 mm below the midpoint. Roughness average (R_a) values were computed for each profile. Porosities were formed on some of the samples, due to the properties of the material. The calculation of R_a was made so these porosities were excluded. For some of the samples it was also possible to compute the volume of the removed material. To find an initial value for R_a, prior to brushing, R_a was also computed from parts of each profile that were outside the abraded area. All profiles started and ended outside of the abraded area.

RESULTS

PAPER I

The results are shown in Table 4 [Figures showing correlations between R_a versus volume, RDA versus volume and RDA versus R_a, go to original paper I at the end of this book]. Three samples of each toothpaste were analysed. The volume and the R_a measurements are presented for each toothpaste along with the SD, and also the RDA-values received from the manufacturers of the toothpastes. As can be seen in Table 4 and [Fig. 7 in the paper I at the end of the book, the correlation between the RDA-value and the R_a-value was very low ($R^2 = 0.04$)], with toothpaste A displaying a low RDA-value (40) and the highest R_a-value (5.73), while toothpaste F is showing the highest RDA-value (130) and a R_a-value of 1.84. Toothpaste K has the lowest RDA-value (30) and a R_a-value of 1.13. [Figure 6 in the original paper I at the end of the book, illustrates the correlation between RDA and volume measurements which also was low ($R^2 = 0.00002$). The correlation between R_a and volume measurements is illustrated in Fig. 5, and was found to be good ($R^2 = 0.87$, P = 0.003%)], where toothpaste D show the lowest R_a-value and the lowest volume measurements. R^2 designates the square of the Pearsons correlation coefficient.

Table 4: Results of the toothpastes abrasion measurements. For each toothpaste the roughness average (R_a), abraded volume, as well as the radioactive dentin abrasivity (RDA) value is shown. SDs for R_a and volume have been calculated.

Toothpaste	R_a (μm)	SD	Volume (mm^3)	SD	RDA
A	5.73	0.61	9.27	0.50	40
B	4.83	1.25	9.68	0.26	40
C	0.83	0.34	3.48	0.30	70
D	0.56	0.12	1.39	0.18	50
E	1.08	0.25	3.27	0.24	55
F	1.84	0.30	5.57	0.32	130
G	1.58	0.33	5.86	0.34	79
H	1.23	0.32	3.25	0.19	50
J	1.27	0.26	2.70	0.83	55
K	1.13	0.39	3.21	0.13	30
L	0.87	0.32	3.92	0.25	50

PAPER II

In tables 5 and 6, the R_a-values and the volume loss values obtained from the different toothbrushes used with water alone and with Clinomyn® are presented. In Table 5, the R_a-values after 1- and 6-h brushing are presented together with the respective standard deviations. In Table 6, the volume loss values after 1- and 6-h brushing are presented in the same way. The toothbrush that caused the highest R_a-value, i.e. the highest roughness value on the acrylic plates after brushing with water, was Jordan Medium® both after 1 and 6 h ($P < 0.0001$), and the toothbrush causing the least abrasion was TePe select® and Dentosal® after 1 h ($P < 0.001$) and TePe select® and TePe x-soft® after 6 h ($P < 0.001$). After brushing for 1 h with Clinomyn® toothpaste, Butler Gum® showed the highest Ra-value ($P < 0.0001$), i.e. greatest abrasion, and TePe select® and TePe x-soft® the lowest, ($P < 0.001$). The 6-h brushing with toothpaste (Clinomyn) also revealed that Butler Gum® caused the highest abrasion ($P < 0.0001$), and Pepsodent® the lowest, ($P < 0.0001$).

Concerning the quantitative values (volume loss), Jordan Medium again showed the highest values after 1 h with Clinomyn and TePe select the lowest, however, not significant against all the other brushes. After 6 h with Clinomyn, Butler Gum revealed the highest volume loss values followed by Jordan Medium, and the lowest 6 h values were shown by TePe select and OB Cross A, however, not significant against all the other brushes. The volume loss values caused by abrasion from water alone had such a high uncertainty that ranking was not possible neither for one or 6 h of brushing; therefore, no SD values were relevant. [Significance of differences (p-values), go to paper II at the end of this book].

The correlation of R_a and volume loss values with filament diameter, number of filaments and abrasion values, after 1 and 6 h, are shown in Table 7. Here, it can be seen that after 1 h of brushing, the R_a-values decreased with increased number of filaments ($r = -0.295$), while there is no dependence of the total brushing area. The volume loss values also decreased with increased number of filaments ($r = -0.388$), but there was a weak dependence of filament diameter and a decrease with the total brushing area ($r = -0.446$). After 6 h, the R_a-values still decreased with increasing number of filaments, but the volume loss values showed a small increase. The R_a-values showed furthermore a small increase with increasing filament diameter ($r = 0.228$), but the volume loss values had no dependence at all. Regarding the total brushing area, the R_a decreased with increasing area ($r = .0.333$), and volume loss showed a small non-significant increase.

Table 5: R_a (roughness average) values (μm) for toothbrushes using water and Clinomyn

Water 1 h		±SD	Water 6 h		±SD
Jordan Medium	0.344	0.07	Jordan Medium	0.456	0.079
OB barn	0.140	0.015	OB barn	0.235	0.042
Pepsodent	0.094	0.021	OB cross A	0.184	0.028
Butler Gum	0.086	0.02	Dentosal	0.151	0.021
Jordans soft	0.083	0.015	TePe vågig	0.151	0.015
TePe vågig	0.075	0.015	Pepsodent	0.120	0.015
TePe x-mjuk	0.072	0.015	Butler Gum	0.119	0.032
OB cross A	0.069	0.011	Jordan soft	0.097	0.008
Dentosal	0.05	0.005	TePe select	0.082	0.014
TePe select	0.049	0.013	TePe x-mjuk	0.069	0.013
Clinomyn 1 h			Clinomyn 6 h		
Butler Gum	3.223	0.514	Butler Gum	17.433	0.3197
OB barn	1.409	0.334	Jordans soft	14.542	1.549
Jordan Medium	1.281	0.143	TePe select	11.198	2.554
TePe vågig	1.271	0.086	TePe vågig	9.247	0.761
Jordan soft	1.198	0.078	OB barn	9.091	2.813
OB cross A	1.086	0.124	TePe x-mjuk	8.696	2.168
Dentosal	0.883	0.165	Dentosal	8.340	1.352
Pepsodent	0.747	0.121	Jordan Medium	7.572	0.914
TePe select	0.666	0.124	OB cross A	5.601	0.604
TePe x-mjuk	0.597	0.119	Pepsodent	3.645	0.749

Table 6: Volume loss values ($mm^{3)}$ for toothbrushes using water and Clinomyn. Values for brushing with water have a high uncertainty; therefore, SD values are not shown.

Water 1 h			Water 6 h		
Jordan Medium	0.15		Jordan Medium	0.28	
Pepsodent	0.14		OB barn	0.21	
OB barn	0.11		OB Cross A	0.14	
TePe vågig	0.09		Butler	0.13	
Butler	0.07		Dentosal	0.11	
OB Cross A	0.07		TePe vågig	0.09	
TePe x-mjuk	0.05		Jordan soft	0.08	
Jordan soft	0.05		Pepsodent	0.08	
TePe select	0.03		TePe x-mjuk	0.08	
Dentosal	0.02		TePe select	0.03	
Clinomyn 1 h		±SD	Clinomyn 6 h		±SD
Jordan Medium	0.97	0.19	Butler	6.2	2.23
TePe vågig	0.96	0.1	Jordan Medium	5.45	1.14
Butler	0.88	0.2	TePe vågig	5.32	0.34
Jordans soft	0.75	0.14	Dentosal	5.28	1.88
Pepsodent	0.74	0.35	TePe x-mjuk	4.98	4.05
TePe x-mjuk	0.67	0.55	Jordan soft	4.95	2.09
OB barn	0.65	0.06	OB barn	2.91	0.87
Dentosal	0.48	0.24	Pepsodent	2.82	2.48
OB Cross A	0.33	0.18	TePe select	2.68	3.26
TePe select	0.31	0.15	OB Cross A	1.63	0.11

Table 7: Correlations between R_a and volume loss with number of brush filaments, filament diameter and brush area. Pearson´s correlation test was used.

	Number of filaments	Filament diameter	Brush area*
After 1 h brushing			
R_a	–0.3	0.53	0.06
Volume loss	–0.39	0.22	–0.45
After 6 h brushing			
R_a	–0.33	0.23	–0.33
Volume loss	0.22	0.07	0.16

*Brush area = number of filaments times area of one filament.

PAPER III

The results are presented in three tables. Since all samples had different roughness (R_a) initially, we decided to compute the ratio between the initial roughness and the roughness of the abraded area for each sample. The average of the ratios for each pair of samples are presented in Table 8. The highest ratio were obtained for Tetric Ceram after brushing with Pepsodent for six hours, and slightly lower after one hour. This small change with time would indicate that this material is the most resistant to abrasion. However, these values are uncertain since the difference between the plates in these pairs was quite high, hence the large standard deviation.

For some materials the ratio decreased with time, indicating a polishing effect.

This effect could be seen for Dyract Flow brushed with Colgate and Pepsodent. Tab 2000 showed no change in R_a (within error limits) for Colgate and quite a large change (0.7 to 12.1 ratio) for Pepsodent which would indicate that this is one of the materials least resistant to abrasion. Negligible difference were shown for water, i.e. no abrasion could be observed.

Table 9 and 10 show the specific R_a values for unbrushed and brushed materials after 1 and 6 hours of brushing. Table 9 shows data for Pepsodent Whitening and table 10 for Colgate Smiles. A polishing effect could be seen in some cases, i.e. the roughness was lower in the abraded area than in the unbraded. This can be seen in the R_a values as well as when inspecting the profiles. The abraded areas had in these cases a smoother appearance, Figure10.

Table 8: The ratio of R_a for abraded parts to R_a for un-brushed parts of the profiles.

	Colgate		Pepsodent		Water
	1 hr	6 hrs	1 hr	6 hrs	6 hr
Carisma	2.0	6.4	7.4	10.6	1.0
Dyr	2.6	1.7	7.2	5.7	1.5
T Ceram	6.9	5.8	18.0	21.2	1.4
Tab	1.3	1.0	0.7	12.1	1.2
Grandio	3.6	4.4	6.1	10.1	0.9

Table 9: Roughness average values (R_a) for all materials brushed with Colgate for one and six hours. All values in µ-meters.

	Colgate			
	1 hr		6 hrs	
	intial	abraded	intial	abraded
Carisma	0.013	0.026	0.012	0.076
Dyr	0.021	0.060	0.018	0.029
T Ceram	0.039	0.165	0.015	0.086
Tab	0.444	0.554	0.563	0.541
Grandio	0.012	0.044	0.016	0.068

Table 10: Roughness average values (R_a) for all materials brushed with Pepsodent for one and six hours. All values in µ-meters.

	Pepsodent			
	1 hr		6 hrs	
	intial	abraded	intial	abraded
Carisma	0.012	0.092	0.033	0.362
Dyr	0.015	0.111	0.020	0.114
T Ceram	0.011	0.180	0.187	1.833
Tab	1.772	1.213	0.581	7.024
Grandio	0.017	0.089	0.016	0.169

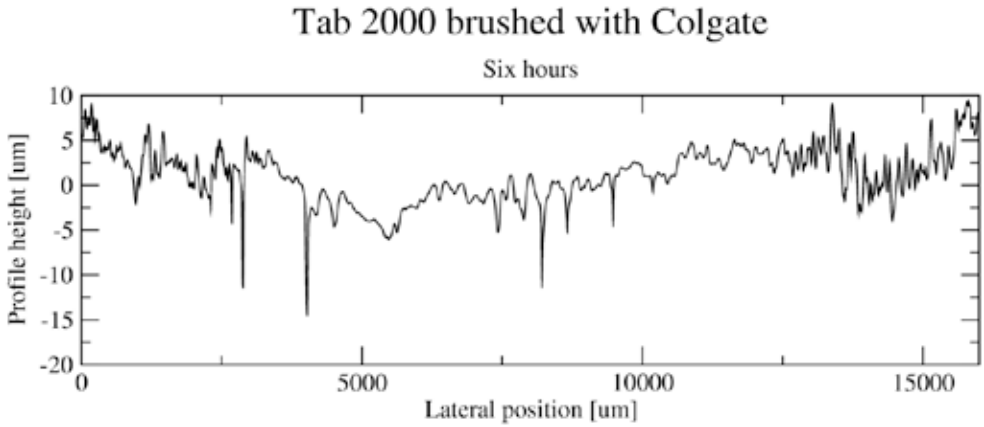


Figure 10: Example of combination of material and toothpaste that gives a lower surface roughness (R_a) after brushing. Center part of profile is smoother, i.e. has less small features, than the left and right ends of the profile, which were outside of the brushed area. Some porosities can also be seen as deep, sharp depressions.

DISCUSSION

The present thesis not only presents a unique technique for measuring abrasivity of both toothpastes and toothbrushes, it also relates these findings both to wear of dental materials and bacterial accumulation. The uniqueness of the profilometer used in the first study is obvious, where it is shown that it is possible to measure the qualitative aspect of abrasivity i.e. the roughness of the abraded surface, measured by the R_a-value (mean arithmetic value) and also, through mathematical analysis, the quantitative aspect i.e. how much of the surface that has been abraded (volume-loss). We have hereby challenged the radiotracer method (RDA-value; radioactive dentin abrasivity value) that for many years has been the "gold standard" when measuring toothpaste abrasivity. Hefferren et al., (1984) stated that the radiotracer method was more precise than the profilometric method. However, since then the profilometers have developed rapidly and are today very precise instruments, which measure at Angstrom level, as described in the materials and methods section. Compared to the weaknesses of the radiotracer method e.g. the heterogeneity of the dentin specimens, the profilometer is today superior.

The dental materials that have been investigated in study III are commonly used in clinical dentistry all over the world. The importance of investigating how they are affected by toothbrushing with toothpastes is limitless since surface roughness is strongly correlated to bacterial accumulation and discoloration. The esthetic appearance of the teeth shows a worldwide growing interest. One aspect of this is whiter teeth - important to most people - probably as a health indicator.

In paper I-II we have exclusively used the brushing machine and the profilometer to calculate the abrasive effect of toothpastes and toothbrushes. We have applied this knowledge in study III and investigated the abrasive effect of toothbrushing with toothpastes on dental materials.

Wülknitz, (1997) studied the correlation between the cleaning power of 41 different European toothpastes and the dentin abrasion. The cleaning power was measured with the pellicle cleaning ratio (PCR) method, which was defined as the ratio of the increase in brightness of the tooth specimens brushed with the test paste divided by the increase of the (calcium pyrophosphate) reference. The dentin abrasivity was measured with the RDA method. The correlation was found to be low and these results were explained by the different influence on dentin and stains by factors such as abrasive type, particle surface and size and also the chemical influence of other toothpaste ingredients. This finding is partly in line with the findings in the present study (I) where we found a very low correlation between the RDA-value and the R_a-value, which means that a toothpaste can exhibit a high RDA-value but still create a smoother surface than a toothpaste with a low RDA-value. Barbakow et al., (1987) showed that chemically different types of abrasives

can have different cleaning/abrasivity patterns and also that chemically identical abrasives such as hydrated silica or calcium carbonate can differ distinctively in these matters and can also have different cleaning/abrasivity ratios. Moore et al., (2005) showed that toothbrushing with water alone appeared to remove the smear layer only, while the detergents (note, not the abrasives) exceeded the predicted smear layer thickness, hence starting to abrade dentine. Three different silica abrasives differed in abrasion properties despite similar particle size distribution. Different detergents modulated the abrasive actions in both positive and negative directions.

It is difficult to distinguish between the effect of the toothbrush on the abrasivity from that of the toothpaste, and it is probably dependent on the interaction between them (Dyer and Addy, 2000). During the years, the toothbrush has only been considered to contribute to tooth surface abrasion indirectly through harboring the toothpaste across the surface and in itself only having a negligible effect (Harrington and Terry, 1964; Absi et al., 1995). The hardness of the filaments is regarded to have a certain influence on gingival retraction (Smith, 1995; Addy, 1998); however, long-term studies are inconclusive, and the prevalence of recession is dependent on the age and characteristics of the population. Nevertheless, toothbrush abrasion may be an integral part in the etiology of recession (Litonjua et al., 2003). A well-spread opinion is that hard toothbrushes cause more abrasion than soft brushes; however, there are only a few studies to support this (Harrington and Terry, 1964; Skinner and Takata, 1951). There are also studies supporting the opposite, i.e., that soft brushes lead to more abrasion than hard ones (Phaneuf et al., 1962; Dyer and Addy, 2000). This is to some extent supported in the present study (II) where we concluded that a softer toothbrush may cause equal or even more abrasion than a harder one. This is explained by the fact that soft bristles are able to retain more toothpaste and creating a larger contact surface onto the substrate. However, a larger surface contact should also mean a better cleaning ability. Other researchers claim that the filament hardness of the toothbrush is not a factor that influences abrasivity (Björn and Lindhe, 1966; Bergström and Lavstedt, 1979).

In study II we measured the number of filaments as well as diameter and length. The purpose was to try to interpret other authors results in that matter. Nygaard-Ostby et al., (1979) pointed out that a simple method for measuring stiffness of toothbrushes is considered relevant and that the stiffness and not the hardness is the proper expression since it must be understood as a deflection force and not hardness of a material. They further stated that the stiffness depends on the bristle material, the diameter and length of the bristles, number of filaments per tuft, number of tufts, positioning of the tufts, bristle design (profile), humidity

and temperature when used in the mouth. Yankell et al., (2001) examined round bristles divided into three diameter groups 5 mils = 0.127 mm, 6 mils = 0.152 mm and 7 mils = 0.178 mm. They stated that the wider diameter the stiffer toothbrush and also that the deposit removal depth was improved with wider diameter. However smaller diameter gave better access to interproximal areas. In our study, Butler gum 431® and TePe vågig®, soft had a diameter exceeding 7 mils. According to the manufacturers all were in the range of soft toothbrushes. Another study focused on the bristle end rounding. This because of the risk for trauma from tooth brushing caused by sharp and edged bristle end geometries (Jung et al., 2003).

When applying this knowledge on dental materials we found that most composite materials were influenced by toothbrushing with toothpastes. However, the toothbrush itself did not cause any wear on any of the dental materials tested which also other authors have found (van Dijken et al., 1983). Tanoue et al., (2000) tested the wear resistance of seven different composite materials and found that the results varied regarding wear and surface roughness. In our study, we found that the R_a values for most of the composite materials increased between 1 and 6 hours of brushing, indicating that the surface became rougher. This might be explained by the fact that when brushing on the composite materials the resin material wears away leaving the large filler particles sticking up from the surface, which also is in line with results from van Dijken et al., (1983). However, on some of the materials a decrease in the R_a-value was found between 1 and 6 hours of brushing, indicating that the surface in these cases had become smoother i.e. a polishing effect was obtained. The influence of the toothpaste was also studied and it was shown that after using the whitening toothpaste Pepsodent, higher R_a values were obtained, i.e. a rougher surface. This result has a direct impact on the clinical situation, where on the one hand the goal is to take away plaque and discoloration but on the other hand it is important to achieve a surface that is smooth and not likely to attract new discoloration.

In a study by Frazier et al (1998), the wear resistance of different resin-based composites and compomers were compared, and they found that all but one hybrid resin-ionomer type material exhibited a resistance to toothbrushing with toothpaste that was as good as or better than that of the traditional resin-based materials. However, they only measured mass-loss after 120000 strokes. In the present study mass or volume loss was not investigated since we considered the surface roughness of greater importance.

In most of the in vitro abrasivity studies performed, some kind of brushing machines have been used. (See chapter Brushing machines). To be able to understand the clinical significance of these studies various attempts have been made to translate these results into a clinical reality. The following authors have extrapolated their results into corresponding brushing time in real life:

1951	Sexson & Phillips:	20000 strokes = 2 years of twice daily brushing
1969	Wright	20000 strokes = 2 years of twice daily brushing
1976	Heath&Wilson	20000 strokes = 2 years of twice daily brushing
1984	Hengchang	25000 strokes = 3-7 years of twice daily brushing
1985	Jones	60000 strokes = 1,6 years of twice daily brushing
1985	Staffanou	75000 strokes = 4,5 years of twice daily brushing
1987	van Dijken	8000 strokes = 1 year of twice daily brushing

One of the reasons for the different estimations is the heterogeneity of the toothbrushing machines, some with circular and others with straight movements.

PAPER IV

MICROBIOLOGY PART. MATERIALS AND METHODS

ADHESION AND BIOFILM FORMATION

Adhesion - in order to study the role of glucosyltransferases and glucan formation on primary adhesion of S mutans IB to titanium specimens these were preincubated at 37° C with 2% sucrose and culture supernatants from glucose-grown cultures (control) of S mutans IB containing glucosyltranferase for 2h.
Biofilm assays - the titanium specimens were incubated in duplicates with washed cells in 2.0 ml BHI with either sucrose or glucose for four hours at 37º c. Washed cells prepared as described above were incubated in BHI (Brain Heart Infusion) medium containing either glucose or sucrose and incubated for four hours at 37º C. Desorption of bacteria and viable count was conducted as described above.

The same Profilometer and set up was used as in studies I-III to evaluate the different degrees of roughness of the titanium plates. The titanium specimens was subjected to brushing in the brushing machine for 12 000 double strokes. Since no abrasion was detected on the specimens, roughness was increased by sandblasting and decreased with polishing equipment.

Figure 11: *Aluminum oxide 250 µm*

Figure 12*: Polierpaste*

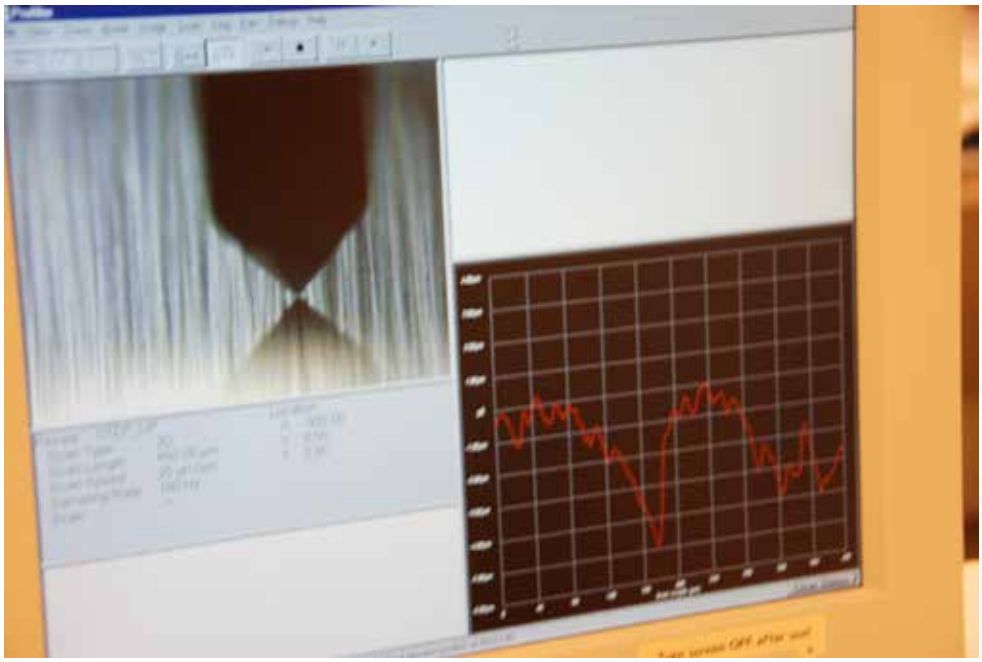


Figure 13: *To the left: the diamond stulys scanning across the the abraded surface. To the right: Profile under development in the computer.*

Titanium specimens and surface treatment:
Six flat square machined titanium specimens, 10 x 10 mm, with a thickness varying from 1.1 to 1.3 mm, was obtained from an implant company (Nobel BioCare®). Two specimens were polished using sanding sheets: SIA® 1951 saiwat P280, 3M® Wetordry Tri-M-ite P400, P600, P1200 used in this order. Subsequently, we applied 3M Imperial Lapping Film grade 12, 3, and 0.3 micron. Finally, Polierpasten-Riegel, PP4 HGP from Pferd® was used to obtain a mirror or glossy appearance (Fig 14). Two specimens retained their original (machined) roughness (Fig 15) and two specimens were sandblasted with 250 µm aluminum oxide particles from Simed for 7 minutes on each side (Fig 16).

Fig14
(μm)

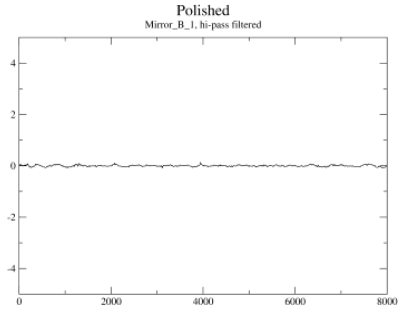


Fig 15
(μm)

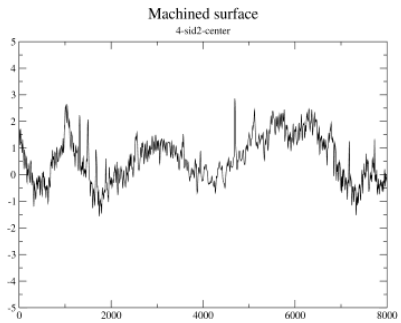
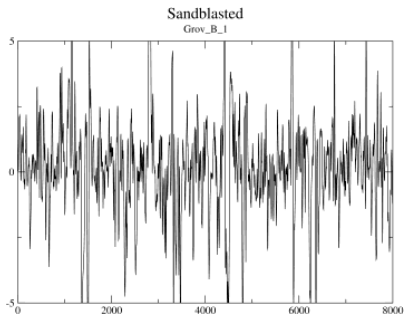


Fig 16
(μm)



All six were identical on both sides. The specimens were washed, using a ultrasonic bath for 20 minutes in distilled water and then in 96% ethanol.

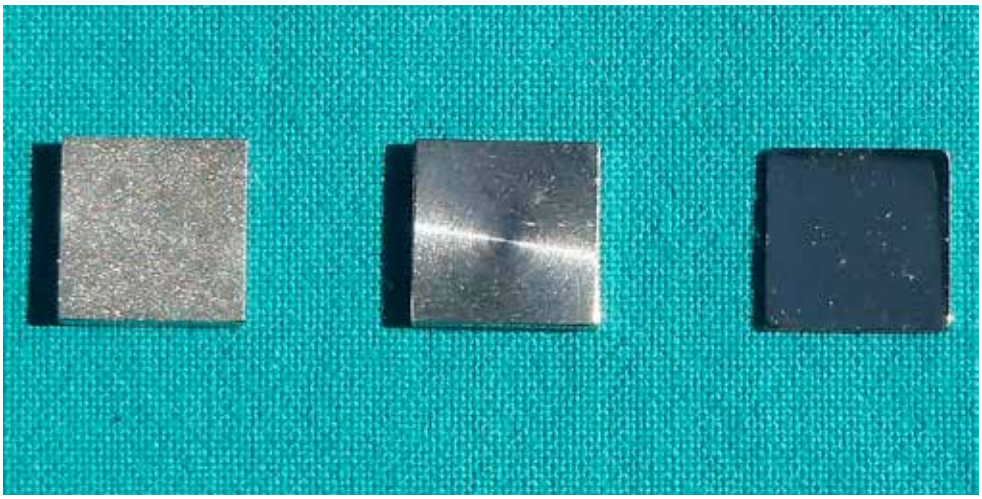


Figure 17: *Three degrees of roughness from left to right; sandblastered, original, mirror glossy.*

Bacterial strains and cultivation:
Streptococcus mutans Ing Britt (IB), a serotype c strain was obtained from lyophilized ampoules and precultures were grown for 16 h at 37°C in Brain Heart infusion broth with 1% glucose (BHI). In order to obtain late exponential phase cells, a 1-ml portion of the preculture was used to inoculate 100 ml BHI medium containing 1% of either glucose or sucrose as energy source and grown for 8-10 h at 37°C. The bacteria were sedimented by centrifugation (10 000g, 10 min at room temperature) and washed three times in cold 0.02M PBS pH 7.2 and then homogenized with a glass tissue grinder equipped with a teflon plunge (A.H. Thomas, USA) and used without delay in the adhesion or biofilm assays. Composition of the BHI medium: Brain Heart Infusion Broth (BHI) (Difco) 4.0 g, FMC medium 0.5 g, cysteine 0.1g, K_2HPO_4 0.2 g, NaH_2PO_4 0.04 g and glucose or sucrose 1,0 g and distilled water to a final volume of 100 ml.

Adhesion assays:
Washed bacteria were transferred to 0.02M PBS pH 7.2. The cell concentration was 2.1±0.3 x 10^9. The three types of titanium specimens as described previously were immersed in duplicates into 2.0 ml freshly prepared cell suspensions in glass tubes and incubated at room temperature for 2 h. The titanium specimens were then washed three times in 10 mM PBS pH 7.2 and subsequently transferred to 2.0 ml 0.02M PBS pH 7.2 and sonicated for 5 min at room temperature (Elmasonic S, Elma GmbH & Co KG, Singen, Germany) and then vortexed for 15 sec to release adsorbed bacteria. The released bacteria were serially diluted and incubated

on mitis-salivarius agar and cultivated anaerobically in a GasPak for 48 h. The number of bacteria was determined as the mean value of three dilutions.

In order to study the role of glucosyltransferases and glucan formation on primary adhesion of *S. mutans* IB to titanium specimens these were preincubated for 2 h at 37° C with a combination of 2% sucrose and culture supernatants from glucose-grown cultures of *S. mutans* IB containing released glucosyltranferases. Titanium specimens preincubated without sucrose were used as controls. The titanium specimens were then washed three times in cold a.d. and then incubated with washed glucose-grown exponential-phase cells for 2 h at room temperature. The cells were desorbed and quantified by the standard procedures.

In order to investigate the effect of experimental saliva pellicle on cell adhesion in some experiments the titanium specimens were preincubated with clarified saliva for one hour in room temperature. The specimens were then washed twice in aqua dest. and immediately used in primary adhesion assays. Stimulated saliva was obtained from two of the authors, pooled and clarified by centrifugation (10 000 g for 15 min).

In some experiments the number of residual bacteria on the titanium specimens after desorption were estimated by a semiquantitative method. The specimen was washed once in a.d. and then pressed against the surface of an MSB-agar plate. After anaerobic incubation at 37° C for 48 h the number of colonies were counted.

Figure 18: *Elmasonic S® for sonication.*

Figure 19: *Vortexing by Dr. Linder.*

Biofilm assays:
The three types of titanium specimens were incubated in duplicates with washed cells in 2.0 ml BHI with either sucrose or glucose for four hours at 37º C. Desorption of bacteria and viable count was conducted as described above.

Statistical analysis:
The significance of the difference in relation to titanium roughness, primary bacterial adhesion, biofilm formation was calculated using unpaired t-test (for calculating equality between means). The mean values were calculated out of six experiments.

RESULTS

The R_a - values of the titanium specimens are shown in table 1, where obvious differences in surfaces roughness can be observed.

Table 11.

TITANIUM SPECIMEN	Plate I, R_a (μm)	Plate II, R_a (μm)
Polished	0.026	0.029
Machined surface (original)	0.489	0.254
Sandblasted	2.12	1.67

Statistical analyses revealed that no difference in relation to titanium roughness could be detected.
Primary bacterial adhesion was significantly stimulated by previous growth in medium containing sucrose compared to growth in glucose medium. $1.1 \times 10^7 \pm 0.7 \times 10^7$ and $9.5 \times 10^4 \pm 0.97 \times 10^4$, respectively ($p < 0.05$). The numbers refer to single titanium specimens.

Biofilm formation conducted in growth medium containing sucrose contained significantly more bacteria $2.07 \times 10^8 \pm 1.97 \times 10^8$ than in the presence of glucose $3.95 \times 10^5 \pm 4.0 \times 10^5$ ($p < 0.05$). The numbers refer to single titanium specimens.

The mean values were calculated out of six experiments.

Primary adhesion to titanium preincubated with glucosyltranferases and sucrose showed higher amounts of bacteria compared to the control. The tendency was strong although not statistically significant ($p<0.058$).
Salivary pellicle did not seem to affect primary adhesion or biofilm formation.

The experiments conducted to determine residual bacteria after the desorption process revealed that 0.5-1% of the amount of desorbed bacteria remained on the specimens.

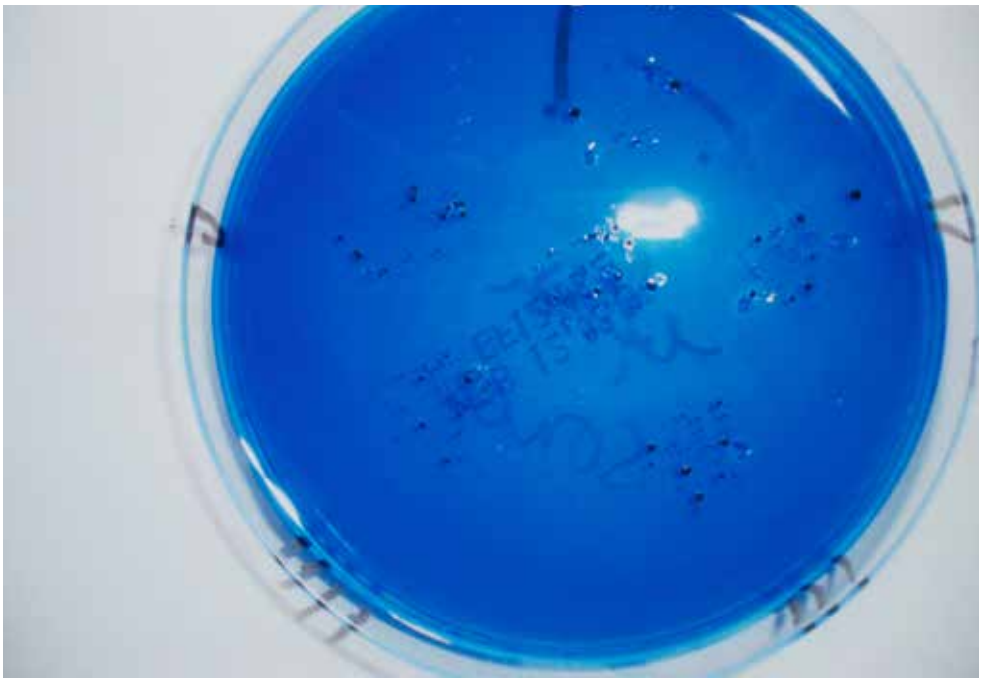


Figure 20*: Experiment to determine residual bacteria from the titanium specimens.*

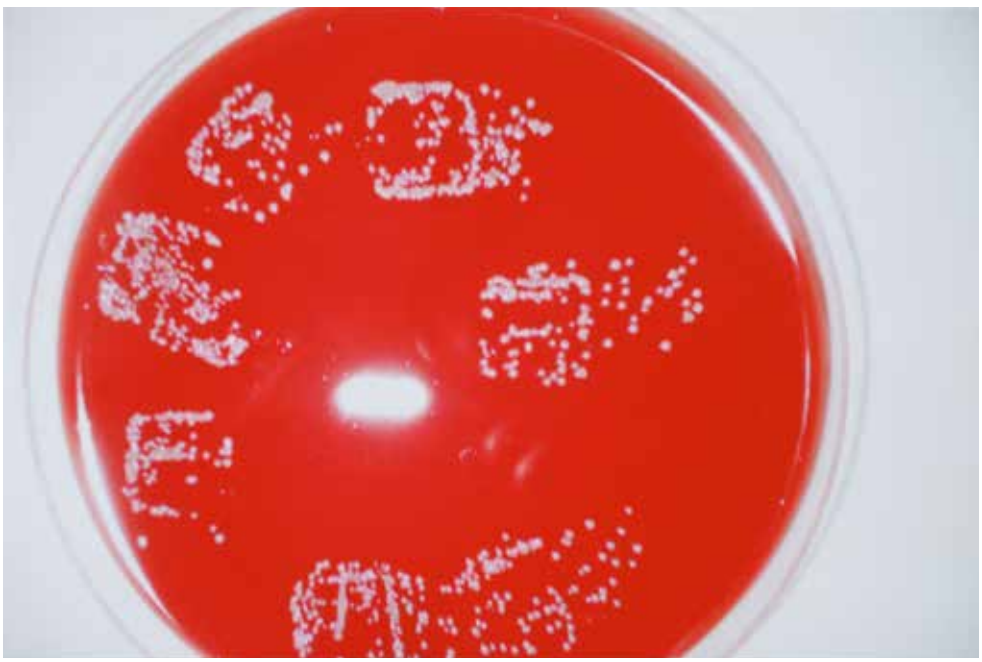


Figure 21: *Primary adhesion experiment with sucrose. Quantification*

DISCUSSION

It is more and more apparent that oral microbes comprise a complex community. Health and disease depends on the interaction between the host and the microbial community as a whole. Although it is important to continue studies of the pathogenic properties of specific microbes, the understanding of the whole microbial community that drive sickness or health in the oral environment plays a key role.(Jenkinson et al., 2005)

Most studies examining the commensal human oral microbiome are focused on disease or are limited in methodology. In order to diagnose and treat disease at an early stage –definition of health is indispensible. By sample from several intraoral nisches in three healthy individuals (dental surfaces, cheek, hard palate, tongue and saliva), Zaura et al 2009 found over 3600 unique sequences, over 500 different species level phylotypes and 88-104 higher taxa. The predominant taxa belonged to Firmicutes (e.g. genus *Streptoccus*), Proteobacteria (e.g. genus *Neisseria*), Actinobacteria (e.g. genus *Actinomyces*), Bacteroides (e.g. genus *Prevotella, Porphyromonas*) and Fusobacteria. The test subjects shared 1660 of 6315 unique sequences. These shared sequences – "the core microbiome" contributed to 66% of the reads. Nearly all reads (99.8%) belonged to the shared higher taxa. This gives an insight into the diversity and uniqueness of individual oral microbiomes, and that a major proportion of bacterial sequences of unrelated individuals are identical. This supports the concept of a core microbiome at health.

When using toothbrush in combination with a toothpaste no abrasion occurred in our study. Starting from a rough titanium specimen R_a = .0254 and 0.489 no alteration was seen. This is in line with Duarte et al (2009). They stated that rough –surface profiles R_a = 0.70 was not altered with laser nor metal or plastic curettes or with an air powder abrasive system. When starting from a smooth titanium specimen R_a = 0.18, contradictory metal curets (and even plastic curets, even though not statistically significant) could easily cause scratches. They did not recommend metal curettes for smooth titanium surfaces. Biofilm formation was evaluated for *S.sanguinis*. Furthermore, rough surfaces treated with metal curettes or the air powder system, were less susceptible to bacterial adhesion due to their specific texture, even compared to smooth surfaces.

Somewhat contradictory to this Hossain et al., (2006) showed that the surface of titanium could be altered by using toothpastes. However they used a brushing machine and brushed altogether 350.000 strokes. To extrapolate these in vitro results into a clinical reality this would correspond to approximately 20 years of toothbrushing twice daily (van Dijken and Ruyter, 1987).

The three different R_a values obtained in the present study corresponds to a mirror glossy appearance (R_a = 0-026 resp 0.029), a medium rough surface (0.489 respective 0.254) and a sandblasted rough surface (2.12 respect 1.67).

The correlation of these abrasivity studies to bacterial accumulation is also obvious. The purpose to choose titanium in the present study was that titanium is almost exclusively used as implant material all over the world. The impact of the investigation regarding the relation between surface properties of titanium and bacterial accumulation is huge. The costs for implants in dentistry for the individual and society at large are enormous. If the risk for failures and loss of implants can be reduced, savings can be expressed both in time, less suffering for the patients and in economy.

In a metanalysis study performed by Esposito et al., (1997) they found when comparing four different implants systems that one of the systems with a smooth surface showed higher incidence of early failures, though decreasing over time. On the other hand a system characterized by rougher surfaces displayed a lower incidence of early failures, but showed constant or increasing failure rates over time. However, in another system with a rough surface a higher prevalence of late failures, attributable to chronic bacterial infection (peri-implantitis) was observed.

Interestingly Bollen et al., (1997) showed that below the roughness value R_a =0.2 µm no further reduction in bacterial accumulation could be expected for teeth, abutments, gold, amalgam, acrylic resin, resin composites and ceramics. Titanium was not involved. Quirynen et al., (1996) showed that a reduction in surface roughness of titanium less than 0.2 µm had no major effect on the microbiological composition, supra- or subgingivally.

The main purpose with study IV was to figure out the *S. mutans* IB adherence and biofilm formation to three different titanium surfaces in terms of roughness. *S. mutans* is one of the major bacteria in the early biofilm formation. Our findings suggest that bacterial accumulation is not likely to be influenced by the surface roughness.

In the early stage after implant insertion no periodontal pathogens are seen in the sulcus fluid during bacterial colonization. Heuer et al., (2007). Almaguer et al., (2012) reported that in the initial biofilm formation titanium roughness plays a minor role. Furthermore they found that hydrophilicity was less important than roughness in the formation of supra-gingival plaque.

Heuer et al., (2011) stated that early implant failures is caused by inflammation of peri implant tissues and that supra and subgingival (mucosa) biofilm is considered to be responsible. They also suggested that implant abutments represents a very valuable approach to study biofilm development. This can be assessed atraumatically by single strand conformation (or chain) polymorphism (SSCP) analysis and subsequent sequence analysis.

Gasik et al., (2012) pointed out that the complications associated with implants are mainly caused by staphylococci, streptococci, Pseudomonas spp. and coliform bacteria. Although increased hydrophilicity of titanium surfaces is known to be beneficial in minimizing biofilm development it has been scarce in quantitative analyses results. Metanalysis, have showed some key factors that could decrease biofilm formation up to 80-90 % by optimizing surface properties and thereby reduce infection risk (Gasik et al., 2012).

Peri-implantitis may develop earlier around implants with rough surfaces, and it may represent a true infection, but the microbiological results failed to fully correspond to the severity of the disease in terms of magnitude. Both culturing and checkerboard analysis were performed in a study by Charalampakis et al., (2012), where they claimed that, microbiological sampling methods should be improved and uniformed.

Thanks to the gene technique it has been shown that *S. cristatus* inhibits expression of fimA, a gene encoding the major subunit of long fimbriae in *P. gingivalis*; as a result , *S.cristatus* interrupts formation of *P.gingivalis* (Bing-yan Wang et al., 2009). The bacterial strains were grown from frozen stocks in Tryticase soy broth (TSB) or on blood agar plates. The RNA in the supernatant was then purified and tested in a Bioanalyser. It seems that culturing technique and the DNA/RNA technique will go hand in hand for many years.

Yet, it must be stated that to ensure optimal peri-implant health the patient must maintain daily biofilm removal with the toothbrush, toothpaste, dental floss and interproximal brushes, and visit the dental hygienist on a regular basis. Keeping the early microbial accumulation to a minimum around the implants and at least eliminate 85 % of the plaque biofilm daily is crucial for long-term success. Kracher et al., (2010).

In the present study *S. mutans* IB was used, the reason was that this bacteria plays an important role in biofilm formation, and also that it in earlier studies has shown to adhere well to solid surfaces (Leonhardt et al., 1995; Hamada and Hutton, 1980).

In other in vitro studies different bacteria have been used e.g. Fröjd et al., (2011) compared biofilm formation by *S. Sanguinis* and *A.Naeslundii* on three different surfaces and found no differences.

Regarding the influence of saliva, the present study did not show any additional effect on bacterial adhesion.
Fröjd et al., (2011) found a significantly greater biofilm volume, when saliva was added on three different titanium surfaces. The contradictory results could be due to the different bacteria used, namely *S. sanguinis* and *A. naeslundi*.

Contradictory results have been found by Lima et al., (2008) who stated that pre-coating of titanium surfaces with experimental salivary pellicle, did not affect the adherence of *A.naeslundi*. However, in that study, the bacteria were suspended in nutrient broth and not in saliva which can be one explanation for the different results.

The significance of the dilutions was also investigated in the present study, where dilution 10^5 did not show any growth; adhesion or biofilm formation, while dilution 10^9 showed a degree of both adhesion and biofilm formation on the titanium specimens.

In our experiments the initial cell concentration was found important both in primary adhesion and in biofilm formation. At initial cell concentrations less than 10^7 the amount of adhering bacteria was relatively low. Furthermore the amount of adhering bacteria was not proportional to the initial cell concentration. We therefore used initial cell concentrations of about 10^9.

In vitro studies on the adherence of *S. mutans* to various solid surfaces have shown the dependence on active synthesis of water-insoluble glucan (Hamada and Hutton, 1980). Sucrose dependent firm adhesion of *S. mutans* to glass has shown to require simultaneous de novo synthesis of water insoluble glucan by glycosyltranferases (Koga et al., 1986). This is to a certain extent supported in our study where the presence of sucrose yielded more bacterial growth than when only glucose was added.

LIMITATIONS FOR STUDIES I-IV

Limitations in studies I and II can be that the brushing trials were carried out on acrylic plates. The reason for choosing acrylic plates instead of dentin specimen was to get a homogenous surface with the same hardness as dentin that would be equal for all the experiments. Furthermore, we only claim the relative comparisons between the toothbrushes and toothpastes.

The filling materials were handled like in the clincial situation, to mimic the ”real situation”. Disadvantage with this method could be that airbubbles occure.

Another limitation is that only bacterial culturing was performed in study IV. But the advantage is that quantification can be done. The method is cost effective and a more austere laboratory environment can be set up and work well. Since we started the study the sequence analysis techniques have developed enormously, but still we have a reliable and exact technique to reasonable costs.

The conventional bacterial and fungi culturing techniques still have their values in detecting and quantifying bacteria and fungi. From a clinical standpoint it gives us rapid results to a low cost. The culturing technique offers a reliable and precise tool for quantification and ability to calculate the mutual relations among bacteria and fungi.

CONCLUSIONS

The RDA-value should be complemented with a qualitative measurement when evaluating the abrasivity of a toothpaste. Furthermore, it is shown that by the use of the results in this profilometer study both the quantitative and qualitative aspects of the abrasivity of a toothpaste can be acknowledged.

The present study showed that the influence of the toothbrush on the abrasivity is negligible when using water as substrate, but when a toothpaste is added, the influence of the toothbrush is of great importance where a softer toothbrush might cause similar or even more abrasion than that of a harder one. Furthermore, one toothbrush–toothpaste combination can cause more volume loss but still create a smoother surface than another that highlights the need for looking at both the quantitative and qualitative aspect when conducting abrasion studies.

The present study showed that the surface of composites was not influenced by tooth brushing with water alone, however when a toothpaste was added, most of the materials exhibited a rougher surface after six hours of brushing than after one hour. On some of the materials a smoother surface was obtained, thus indicating a polishing effect between one and six hours of brushing. It is important to take this into consideration, since a rougher surface attracts plaque more easily and favors discoloration and increases the risk for caries and gingivitis/ periodontitis.

In our study the surface roughness of the titanium plates had no influence on bacterial accumulation. Furthermore, the bacterial growth was more pronounced in the biofilm experiment than in the primary adhesion experiment. The presence of sucrose significantly increased the bacterial growth in the primary adhesion as well as in the biofilm experiment compared to glucose.

FUTURE PERSPECTIVES

To further develop a new method for testing toothpastes and tootbrushes and combinations of these two. The manufacturers and consumers will be offered a cost effective way to test items for oral care.

Roughness differences between the three titanium specimens hade very limited impact on the outcome of *S. mutans* both in the adhesion assays as well as the biofilm formation. This raise the question how other bacteria will react. How would the result have been in a clinical setting? Conventional microbiology assay was performed. DNA evaluation in a clinical situation is possible, even though a quantitative bacterial evaluation is not possible.

Further investigations that have been discussed are to test other bacteria involved in the periodontal disease process. By using conventional microbiology and-or DNA evaluation in in-vivo studies with titanium specimens we will be able to evaluate relationships in the microbiome.

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