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Comparative *in vitro* study of two tooth bleaching systems: colour change and enamel surface effects.

Andries Adam Grundlingh

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Dentistry by coursework and research report. Johannesburg, 2009.

Declaration

I, Andries Adam Grundlingh declare that this research report is my own work. It is being submitted for the degree of Master of Science in Dentistry by coursework and research report at the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination at this or any other University.

_____ **14/09/2009**

Andries Adam Grundlingh Date

Dedication

I dedicate this report to Professor Elly S Grossman. May you accomplish all your dreams and may all your efforts be rewarded.

Presentations arising from this research report

1. Twenty fourth Annual Conference; South African Division, International Association of Dental Research. Midrand, 6-7 September, 2006. Grundlingh AA, Grossman ES. Tooth colour determination using three methods and three tooth types.

2. Twenty fifth Annual Conference; South African Division, International Association of Dental Research. Centurion, 6 September, 2007. Grundlingh AA, Grossman ES. Tooth bleaching results: Ozicure Activator machine and Opalescence 35%. (Awarded the Charles Valcke Prize for the best poster presentation, 6 September 2007).

Abstract

This *in vitro* study compares tooth bleaching and consequences of tooth surface effects of Ozicure Oxygen Activator (O_3, RSA) with Opalescence Quick (Ultradent, USA) tooth bleaching.

One hundred and thirty six teeth (canines, incisors and premolars), which were caries free, had no surface defects and within the colour range 1M2 and 5M3. Teeth were randomly divided into the three experimental groups: Opalescence Quick, Ozicure Oxygen Activator and control. The three experimental groups received three treatments of one hour each over three consecutive days.

Tooth colour was assessed using the VITAPAN 3D MASTER TOOTH GUIDE (VITA, Germany) and VITA Easyshade (VITA, Germany). A randomized block design was used to assess tooth colour change. A General Linear Models test for analysis of variance for a fractional design with significance set at $P<0.05$ was used. Both bleaching methods significantly lightened the teeth, visually ($P<0.0001$) and digitally ($P= 0.013$). Tooth colour change was seen after the first hour of tooth bleaching; thereafter there was little or no benefit. The tooth type played a significant role in tooth colour change (visually $P=0.0416$ and digitally $P=0.004$). The quality of the spectrophotometer may account for the different results found compared to the tooth guide.

Scanning electron microscopy showed no effect of enamel loss following bleaching. Atomic force microscopy showed a 2.5 rougher enamel surface with Opalescence Quick.

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Chapter 1 Background to the study

1.1. General introduction

Patients seek aesthetic treatment to rejuvenate their past youth. Increasing awareness of patients regarding the colour, shape and alignment of anterior teeth, and how these play a major role in facial appearance has driven patients to seek treatment for perceived dental "imperfections". Discolouration of anterior teeth, in particular, is described as leading to low self-esteem, low confidence and a greater awareness of physical or aesthetic imperfection (Scarpelli *et al*., 2008). This has led to the development of a number of aesthetic procedures that specifically target tooth staining, two of which are most used.

Composite or ceramic veneers and crowns improve tooth colour, but entail invasive procedures that result in the removal of sound tooth structure. Additionally it is an expensive and time-consuming procedure. In contrast, bleaching discoloured teeth is a conservative, non-invasive treatment requiring no removal of tooth structure and is an efficient and relatively safe procedure (Sulieman *et al*., 2005). Consequently, tooth bleaching is attracting an increasing number of patients who seek this relatively inexpensive option to create a "bright white smile". So great is the demand for tooth discolouration treatment that it has evolved into an annual multibillion-dollar industry generating a vast amount of published literature.

This is particularly true in the case of tooth bleaching where the literature on tooth bleaching is of varying quality ranging from superficial product promotion to sophisticated, in depth science. This is illustrated by the number of items emerging from an Internet search of two databases when using the terms "tooth bleaching or whitening". A Google search revealed 468 000 strikes while PubMed, using the same terms yielded 4 929 scientific papers.

This has meant that a large quantity of literature needed reviewing for this comparative investigation dealing with traditional tooth bleaching versus a newer ozone based option, Ozicure Oxygen Activator (O_3, RSA). Consequently the literature reviewed had to be divided into two chapters. This, the first chapter, will focus on a broader overview of tooth bleaching and procedures associated with the study. Chapter 2 will cover the literature dealing with the research questions themselves. This has been done to prevent readers of the research report from loosing the thread of the investigation by getting bogged down in necessary theoretical detail.

The sections following immediately will cover basic theory of tooth staining (section 1.2); tooth bleaching (section 1.3) and tooth bleaching side effects (section 1.4). Thereafter the concept of ozone and its use as a tooth bleaching agent will be covered (section 1.5). The final section (1.6) will deal with tooth colour and the two main types of tooth colour assessment.

1.2. Tooth Staining

The crown of a tooth consists of enamel, dentine and the pulp chamber with the dental pulp. A change to any of these components can lead to an alteration in the light transmitting and reflecting properties of the tooth resulting in a change in tooth colour. It is widely accepted that organic compounds, called chromophores are responsible for the change in tooth colour (Dahl and Pallessen, 2003). These molecules do so by altering the light scattering properties of teeth. The causes of tooth staining are varied and complex and can be categorized as intrinsic, extrinsic or internalized.

1.2.1. Intrinsic staining

Intrinsic staining occurs during tooth development and is laid down in the tissue as it is formed. Metabolic diseases such as alkaptonuria or porphyria, systemic factors including inappropriate use of tetracycline or the excessive ingestion of fluoride causes this particular tooth staining. Local factors such as trauma can also result in intrinsic tooth staining. The precise role of chromophores in intrinsic staining is not clear (Watts and Addy, 2001).

1.2.2. Extrinsic staining

Extrinsic staining is found on the tooth surface or in the pellicle and can be either metallic or non-metallic in origin. While the role of chromophores in extrinsic staining is better understood the mechanism of stain formation is highly variable and subject to conjecture (Watts and Addy, 2001). Successful removal of superficial extrinsic tooth stains can be achieved by scaling and polishing (Joiner, 2004), but is ineffective against more deep-seated stains.

1.2.3. Metallic stains

Metallic compounds can interact with tooth surfaces and lead to staining. Metallic stains may be associated with occupational exposure (iron foundry workers) and medicines such as prescribed or over the counter iron supplements (Watts and Addy, 2001).

1.2.3.1. Non-metallic stains

Non-metallic compounds produce stains as a result of their inherent colour. Examples are chlorhexidine mouth rinses and stains caused by tobacco use (Watts and Addy, 2001).

1.2.4. Internalised staining

Internalized staining occurs when an extrinsic stain penetrates the tooth substance. Penetration occurs in enamel defects and exposed dentine. Pigments may become internalised through developmental defects or acquired defects. Acquired defects include tooth wear, gingival recession, dental caries and amalgam restorations (Watts and Addy, 2001).

The foregoing discussion indicates that tooth staining is well documented and the origins of the different types of stain are well understood.

1.3. Tooth bleaching

The first published dental report on tooth bleaching was in 1877 (Chapple, 1877) when a 10% solution of carbamide peroxide was used in a tray as part of an antiseptic for the treatment of gingivitis. It was only in 1989 that the first paper (Haywood and Heymann, 1989) was published describing a mouth guard worn overnight with 10% carbamide peroxide to lighten the teeth.

1.3.1. Mechanism of tooth bleaching

Hydrogen peroxide is the commonly used active agent in tooth bleaching (Kihn, 2007). Hydrogen peroxide can be applied directly to the tooth or is produced in a chemical reaction from sodium perborate (Figure 1.1; equation 1) or carbamide peroxide (Figure 1.1; equation 2). Hydrogen peroxide functions as a strong oxidizing agent. The reaction of hydrogen peroxide with the tooth releases free radicals, reactive oxygen molecules and hydrogen peroxide anions (Figure 1.1; equation 3a). Equations 3b and 3c show the unstable reactive oxygen molecules revert to oxygen and hydrogen peroxide anions.

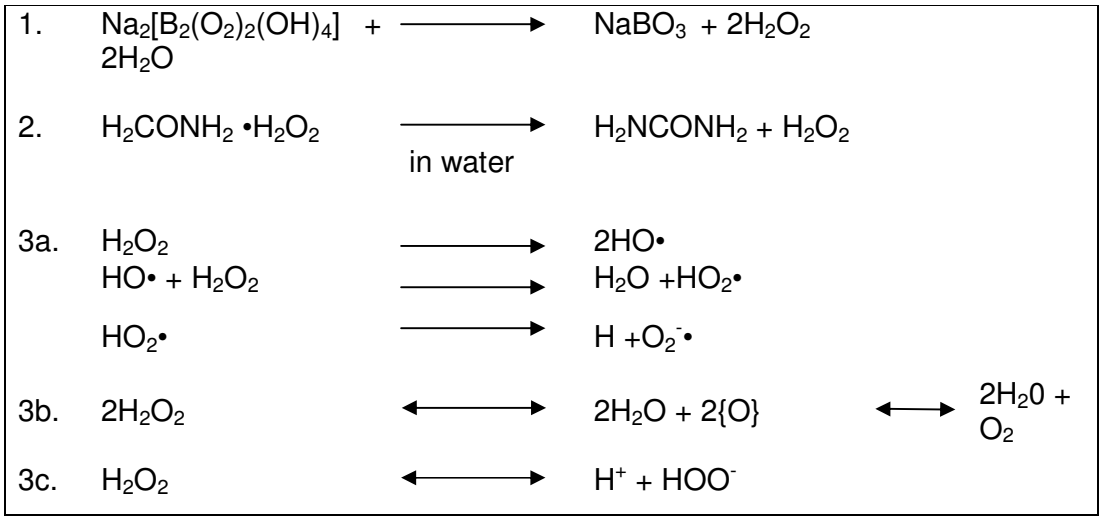


Figure 1.1. Formation of hydrogen peroxide and free radicals from sodium perborate and carbamide peroxide. (from Dahl and Pallesen, 2003)

When carbamide peroxide reacts with water, urea forms as a by-product and can be broken down to carbon dioxide and ammonia. The high pH of ammonia

facilitates the bleaching process, but the amount of ammonia formed during tooth bleaching with carbamide peroxide is unclear (Dahl and Pallesen, 2003). Kihn *et al*., (2000) claim that bleaching agents based on carbamide peroxide produces less of the active agent than hydrogen peroxide based bleaching agents, thereby a less effective bleach. Whatever the source of the active agent, the reactive molecules split the long-chained, dark-coloured chromophore molecules, which are responsible for the stain into smaller, less coloured and more diffusible molecules. This results in a lightening of the tooth.

In summary, the broad mechanism of tooth bleaching, based on hydrogen peroxide as the active agent which oxidizes the chromophores is well understood. However Kihn (2007) points out that the precise mechanism of tooth bleaching remains subject to conjecture.

1.3.2. Types of tooth bleaching

Bleaching agents can be used on vital or non-vital teeth, hence the terms vital or non-vital tooth bleaching (Dahl and Pallesen, 2003). Non-vital tooth bleaching has a completely different approach where a medicament (hydrogen peroxide or sodium perborate) is placed in the pulp chamber, sealed and left for three to seven days. Although the present investigation occurs *in vitro*, it should not be confused with non-vital tooth bleaching. Non-vital tooth bleaching is not part of the study and shall not be further explored. It is only mentioned for completeness.

1.3.3. Vital or external tooth bleaching

There are three basic bleaching approaches today: dentist supervised night guard bleaching, in-office or power bleaching and the “over the counter” bleaching products (Joiner, 2006). These will be discussed in the following section.

1.3.3.1. Dentist supervised night guard bleaching

The dentist supervised night guard bleaching refers to the take home bleaching systems. These systems use low concentration bleaching agent (5% - 22% carbamide peroxide) applied to the teeth with a custom made mouth guard. Manufacturers` instructions vary from twice-daily treatments of 30 minutes to two hours to overnight applications. The major disadvantage of this approach is poor patient compliance (Kihn, 2007).

1.3.3.2. In-office bleaching treatments

In-office treatments utilize 30% to 35% hydrogen peroxide with or without heat or light activation. The gingiva is protected by means of a gingival dam such as OpalDam® (Ultradent, USA). The bleaching agents are applied in the office, the main advantage being immediate visible results. This approach will cost the patient more and multiple in-office visits may be required to reach the optimal result (Kihn, 2007).

1.3.3.3. Light activated treatment

This approach involves the application of a high concentration bleaching agent in-office and a light or heat source to speed up the breakdown of the hydrogen peroxide to create free radicals. Theoretically, this procedure will cause a quicker lightening of the teeth, but there is controversy over the benefit of heat or light activation of the bleaching agents. Wetter *et al.,* (2004) concluded that some of their experimental groups receiving heat or light activation showed a statistically significant difference in tooth colour change. Other studies have indicated no added benefit using light activation sources (Marson *et al*., 2008). The main concern with using heat or light activation is damage to the pulp, especially irreversible pulpitis (Buchalla and Attin, 2007).

1.3.3.4. Combination treatment

Combination treatment involves the use of a high concentration bleaching agent in the office, followed by a five to seven day dentist supervised night guard bleaching treatment. This approach decreases the number of in-office bleaching visits the patient needs (Kihn, 2007).

1.3.3.5. "Over the counter" treatments

Over the counter" treatment options contain low concentrations of hydrogen peroxide (three to six percent). The treatments come in the following forms: toothpastes; mouth rinses; whitening strips; paint on applications and whitening kits with preformed trays. The exposure of the patient's teeth to

these products is minimal and these products have to be used for a long period of time to deliver tooth lightening results. These products have not been extensively studied and inappropriate use by patients raises concerns (Kihn, 2007).

As can be seen many vital tooth bleaching methods are currently available. This present investigation will focus on two tooth bleaching products used as in-office bleaching treatments.

1.4. Tooth bleaching side effects

Tooth bleaching may pose side effects for some patients such as tooth sensitivity and gingival irritation. These side effects can be present during or after vital tooth bleaching and can deter the patient from completing the bleaching treatment.

Bleaching agents are usually applied to the teeth that are visible when one smiles (canine, incisor and premolar teeth). Such teeth have been used in a number of *in vitro* studies (Sulieman *et al*., 2005; Spalding *et al*., 2003), but have been sectioned. The reason was lack of availability of the selected teeth and ease of use during further investigation. Other investigators have utilized molar teeth for their studies, mainly due to the availability of these teeth (da Costa and Mazur, 2007; Efeoglu *et al*., 2007; Worschech *et al*., 2003; Oltu and Gürgan, 2000; Flaitz and Hicks, 1996).

A study by Leonard *et al*., (1998) suggests that different tooth type's respond

differently to bleach. In his investigation canine teeth, responded better to certain bleaching solutions than incisor teeth. Unfortunately, there were insufficient premolars in the sample to draw conclusions for this tooth type.

The present *in vitro* study will mimic the clinical situation by using intact, sound canine, incisor and premolar teeth. It will also parallel the investigation by Leonard *et al*., (1998) to establish whether different tooth types respond differently to a bleaching procedure.

1.4.1. Tooth sensitivity

Tooth sensitivity is usually the most significant side effect experienced by patients (Tam, 1999a). This usually presents as a spontaneous sharp shooting pain limited to one or more teeth (Haywood, 2005), with the adverse effect persisting up to 48 hours after tooth bleaching has been stopped (Tam, 1999b). Jorgenson and Carroll (2002) concluded that half of all patients undergoing tooth bleaching would experience mild sensitivity. Intolerable tooth sensitivity has led to 11% to 14% of patients discontinuing treatment (Leonard *et al*., 1997; Schulte *et al*., 1994).

Published studies have examined diverse aspects of bleach-induced tooth sensitivity. Hewlet (2007) maintains that tooth sensitivity is usually associated with the night guard tooth bleaching approach, while Marson *et al*., (2008) avers that in-office bleaching can produce a higher incidence of tooth sensitivity when hydrogen peroxide is used in combination with heat. Tam, (1999a) reported that although patients experienced tooth sensitivity in incisors

and canines, premolar teeth were never indicated as being specifically sensitive.

In conclusion, tooth bleaching induced tooth hypersensitivity is complex and multi factorial. Although manufacturers have formulated prevention plans by adding three percent potassium nitrate and 0.11 percent fluoride to bleaching agents, da Costa and Mazur, (2007) did not find a statistical significant difference between agent with or with these adjuncts. The mechanism causing the sensitivity remains unknown and consequently the published literature on tooth sensitivity is divided and inconclusive.

1.4.2. Pulp damage

It is widely agreed that the bleach agent penetrates into the pulp and may be a cause of bleach-induced tooth sensitivity (Fugaro *et al*., 2005; Hewlett, 2007). Indeed, Benetti *et al*., (2004) have shown that peroxides diffuse through the enamel and dentine into the pulp chamber following application of bleaching gels containing hydrogen peroxide or carbamide peroxide. Higher concentrations of bleaching agents also produce higher levels of peroxide in the pulp chamber, especially in restored teeth (Benetti *et al*., 2004). Paradoxically the lower concentrations of hydrogen peroxide in whitening strips also cause this problem (Gökay *et al*., 2004).

Tooth bleaching has caused vacuolisation of odontoblasts, predominantly in the pulp horns. Such pulp changes can occur irrespective of the application time of the bleaching agent and are reversible over time (Fugaro *et al*., 2004).

More seriously, permanent pulp damage can result from heat production when bleaching agents are activated with light sources (Buchalla and Attin, 2007).

In theory, the defence mechanism of a healthy pulp tissue would significantly reduce the available levels of free hydrogen peroxide. Gökay *et al*., (2005) suggested that *in vivo* penetrations of hydrogen peroxide might be less than measured in laboratory studies due to positive pulp pressure and osmotic pressure. This they feel, might counter the influx of bleaching gel molecules into the pulp chamber reducing the probability of tooth pain (Gökay *et al*., 2005).

1.4.3. Gingival irritation

High concentrations of hydrogen peroxide (30% to 35%) can burn and bleach the gingiva. Although gingival irritation can be decreased by shortening the treatment time (Haywood *et al*., 1994), Leonard *et al*., (1997) showed that 55% of patients experienced both tooth sensitivity and or gingival irritation. Gingival irritation may lead to patients terminating tooth bleaching treatment.

The above section focuses on the negative side effects of bleaching such as tooth sensitivity and gingival irritation. These painful side-effects may be so great to cause many patients to terminate bleaching treatment. Thus recent research has focused on bleaching procedures which will greatly reduce or eliminate such side effects. A bleaching product has recently come onto the market that specifically addresses a reduction of sensitivity and irritation. It is called Ozicure Oxygen Activator (O_3, RSA), and as its name suggests, this

product relies on ozone to achieve bleaching. The product will be discussed more specifically in section 2.1.3, however, for the purposes of this thesis it is necessary to give an overview of the history of ozone and its use in general health and dental applications. This will be discussed in the immediate sections which follow.

1.5. Ozone – its history and use in health therapy

Ozone is a gas consisting of three atoms of oxygen and previously thought as an acyclic structure. Recently it is believed to have a dative covalent bond. The word ozone comes from the Greek word "*ozein*" and means to smell. A German chemist Christian Friedrich Schonbein was the first to discover ozone in 1840 (Nogales *et al.*, 2008). Ozone is a natural component of the earth and one of the most important gases in the stratosphere. It forms a protective layer that plays an important role in maintaining the biological balance in the biosphere. This gas is produced naturally by electrical discharge from lightening strikes, waterfalls, ultraviolet rays from the sun and crashing surf. A recognizable smell is detectable by the human nose at concentrations between 0.02 ppm to 0.05 ppm. This is approximately one percent of the recommended 15 minute exposure level.

1.5.1. Ozone in health

The first recorded use of ozone for health reasons occurred in the United States of America in 1885 (McCabe, 1994). Ozone therapy is still a controversial topic and has been used experimentally predominantly in

healthcare application in Europe. Ozone gas has been used effectively as an antimicrobial agent against bacteria, viruses, fungi and protozoa. Treatment with ozone gas was been indicated in 260 different pathologies in the medical and dental fields (Nogales *et al*., 2008).

1.5.1.1. Dental application of ozone

In dentistry, ozone has shown enormous potential in a variety of clinical and experimental settings (Stübinger *et al*., 2006). Ozone has been used in the treatment of dental caries; infected intraoral wounds; cleaning dentures containing *Candida albicans* and prevention of dental plaque formation *in vitro* (Nogales *et al*., 2008). Ozone promotes haemostasis, an increase in local supply of oxygen, inhibits bacterial growth and used in the treatment of root caries in elderly patients. Hydrogen peroxide is the fundamental reactive oxygen specie that is produced and can be employed for bleaching purposes (Grootveld *et al*., 2004a).

Ozone is biocompatible with human epithelial cells, gingival fibroblasts and periodontal cells (Ebensberger *et al*., 2002). It appears from the foregoing that the benefits of ozone gas can be exploited successfully at therapeutic levels. However Azarpazhooh and Limeback (2007) caution that the potential of ozone in dentistry can only be fully realized when better designed studies with long-term results are available. They also stress that uses of ozone in the medical and dental fields are dependent on the establishment of well-defined safety regulations, something which is currently absent.

1.5.2. Ozone generators

There are three types of ozone generators for clinical use. Ozone can firstly be produced from oxygen in a narrow frequency bandwidth of ultraviolet light. The term “cold plasma” describes the second type of generator. This involves a device constructed from two glass rods filled with an inert, noble gas excited by high voltage. The voltage jumps between the rods and forms an electrostatic plasma field that converts oxygen to ozone (Nogales et al., 2008). A last method is from a corona discharge involving a tube with a hot cathode surrounded by a screen anode, this is used by the Ozicure Oxygen Activator to produce ozone gas.

1.5.3. Exposure limits, risk factors and side effects of ozone

Ozone can be a toxic gas at high concentration levels and can be fatal at 50 ppm for 60 minutes (Millar and Hodson, 2007). In the United Kingdom the exposure limit of ozone is 0.06 ppm for eight hours per day (five days a week) or 0.3 ppm (0.6 mg/m^3) for 15 minutes (Homes and Lynch, 2004). Hydrogen peroxide and aldehydic products can damage the lungs and extra pulmonary tissues when ozone is inhaled over exposure limits (Grootveld *et al*., 2004b).

Known side effects are rhinitis, coughing, headache, occasional nausea, vomiting, epiphora and upper respiratory irritation. The main problems in the dental and medical fields are the lack of regulation of the use of ozone and the exposure of patients and operators to the ozone gas. Ozone generators do not produce the same percentage of ozone with every application and this makes

it difficult to establish the precise exposure of each generator. However, complications caused by ozone therapy are extremely rare. In cases of intoxication, the patient is placed in the supine position, and humid oxygen is inhaled. The patient is advised to take vitamin E, nacetylcysteine and vitamin C (Nogales *et al*., 2008).

In summary, while therapeutic effects of ozone have been known since the late 19th century it remains an "alternative" therapy and is viewed askance by mainstream health practitioners. No studies have been undertaken on the use of ozone in tooth bleaching making this investigation a first in the field.

1.6. Tooth colour

1.6.1. Evaluation of colour

Colour is described by the Munsell colour space in terms of value, chroma and hue. Value describes the lightness of a colour on a scale ranging from pure black to pure white. Hue presents the different families of colour (examples: red, blue and green). Chroma is the degree of saturation or intensity of a colour (Joiner, 2004; Watts and Addy, 2001). The Munsell colour system is grounded on rigorous measurements of human subject's visual responses to colour putting it on a firm experimental scientific basis. Because of this grounding in human visual perception it is a particularly useful system to use in dentistry where visual colour matching is predominantly used in the chairside situation.

The part of a molecule that is responsible for its colour is called a

chromophore. Chromophores are long chained single and double bonded compounds that absorb visible light and reflect the light to produce the true colour of the molecule. Chromophores are present in both metallic and non-metallic stains (Joiner, 2007).

The assessment of tooth colour will be explored in the next section.

1.6.2. Tooth colour assessment

The traditional method whereby tooth colour is assessed is by visual colour assessment using tooth shade guides and charts. The use of shade guides is highly subjective, but visual colour perception assessment can be improved with training (Watts and Addy, 2001). While dental shade guides have been improved, they do not cover the colour distribution of natural teeth (Ishikawa-Nagai *et al*., 2005). Despite the limitations, tooth shade guides have been used successfully in several tooth whitening studies (Mokhlis *et al*., 2000), and such guides are both quick and cost effective.

None-the-less correctly assessing tooth colour using guides remains a problem for clinicians during bleaching procedures. This is due to three conditions:

This method of tooth colour assessment is dependent on the clinician's ability to discriminate between colours, the light source used, and finally light reflection, refraction and shading.

The tooth itself is problematic for colour assessment. Natural tooth colour is

not uniform; it changes from the gingival margin through the tooth body to the incisal edge. The gingival margin appears darker due to the thinner enamel. The incisal edge appears lighter due to its thinner bucco-lingual thickness, whereas the body of the tooth is somewhere in between (Watts and Addy, 2001).

Consequently, the middle third of the facial surface of the tooth is taken to represent the basic colour of the tooth and this area is used for tooth colour assessment (Cibirka *et al*., 1999; Rosenstiel *et al*., 1991).

The viewing conditions are very important during tooth colour assessment for bleaching procedure purposes. Variables such as the light source, time of day, the surrounding area and the angle at which the tooth is viewed from can affect tooth colour. A tooth assessed under different viewing conditions will display a different tooth colour (metamerism). Thus, viewing conditions have to be standardized before tooth colour is assessed.

Since the 1990s, an increasing number of computer-based instruments for shade selection and determination have become commercially available to overcome inconsistencies and colour mismatch in tooth shade assessment (Kielbassa *et al*., 2009). These are based on the developments in the paint, plastics, printing ink and textiles industries where spectrophotometry and computer calculations based on colour theory have utilised colour science to express colours numerically. Technological advancement has made spectrophotometers and colorimeters more accessible to the dentist and increasingly used to pinpoint the true tooth colour (Joiner, 2004).

The reflected light from an object (in this case the tooth) is emitted by an intense gas-filled tungsten lamp that is integrated into the spectrophotometer. Thus spectrophotometers do not rely on judgment or environmental conditions to evaluate tooth colour, but measure the reflected emission of spectral colours. This ensures that the surrounding light does not influence the measurement (Horn *et al*., 1998). With the use of these instruments, the number of incorrect tooth colour readings is reduced, but tooth colour assessment in a patient's mouth remains difficult (Ishikawa-Nagai *et al*., 2005).

Three previous studies have compared digital with visual colour assessment within a single study (Horn *et al*., 1998; Jarad e*t al*., 2005; Kielbassa *et al*., 2009). All found that tooth colour assessment with a spectrophotometer was more reliable and predictable than using standard tooth shade guides. Essentially the difference between tooth colour matching with visual perception techniques and the use of a modern computer colour matching technique lies in the level of accuracy. Notwithstanding, Kielbassa *et al*., (2009) affirms that in many clinical situations, the human eye is adept at detecting color differences and is the final arbiter of what constitutes a clinically important change. In this chapter a broad overview has been sketched of tooth staining, tooth bleaching and the different types of bleaching. Thereafter the two main types of tooth colour assessment were considered giving the pros and cons of each. The painful side effects of bleaching such as tooth sensitivity, pulpal damage and gingival irritation have been covered.

These side effects have led in turn to the development of an ozone based

bleaching system claimed to counter these side effects. This system is called Ozicure Oxygen Activator (O_3, RSA) and is the subject of this investigation. In the next chapter the literature dealing with the specifics of tooth bleaching and the research questions which arose from the literature review will be covered.

Chapter 2 Review of the literature specific to the study

This study came about as a result of the introduction of a new bleaching product, Ozicure Oxygen Activator (O_3, RSA), which utilises ozone to effect tooth lightening. Nothing has been published about this bleach, therefore a study was conceived to compare the bleaching effect of Ozicure with a clinical bleaching "gold standard". A perusal of the literature on the specifics of such an investigation revealed many "areas of darkness" in the field of tooth bleaching and associated fundamentals. This section of the literature review concentrates on the knowledge base of tooth bleaching, what is known and the "areas of darkness" that led to the ultimate formulation of the research hypotheses this study wished to address.

2.1. Bleaching products

There are presently many bleaching products to choose from on the market. Ozicure Oxygen Activator (O_3, RSA) is used in-office, thus the literature was searched for a "gold standard" as a suitable comparative model for this study.

2.1.1. Factors influencing the choice of a suitable comparative bleaching product

Several practical factors needed to be taken into account, in conjunction with the literature search, in order to select a suitable comparative bleaching product. The product needed to be available in South Africa and in general clinical use as an in-office bleach. It was also necessary for sufficient peer reviewed publications on the product in order for it to be recognised as a valid

gold standard. After an in depth literature review Opalescence Quick (35% carbamide peroxide) was selected as a suitable comparison for the reasons summarized in the next section 2.1.2.

2.1.2. Opalescence Quick (35% carbamide peroxide)

Any new product needs to be evaluated against a gold standard. The bleaching products Opalescence (Ultradent, USA) have been extensively studied and compared to many other bleaching products, nine of which are shown in Table 2.1. These studies range from 1996 to 2007, a further indication of the rigor of the product. Many other tooth bleaches have been investigated and reported on in the literature, but most, after two or three studies, are never mentioned again.

Table 2.1. Tooth bleaches which have been compared with Opalescence and the study reference.

Product	Study Reference
DentlBright (Cura Pharm, USA)	Zalkind *et al*., 1996
Nite White (Discus Dental, USA)	Cibirika *et al*., 1999; da Costa and Mazur, 2007; Hegedüs *et al*., 1998; Turker *et al*., 2003
Nu-Smile (M&M Innovations, USA)	Zalkind *et al*., 1996
Platinum Professional Toothwhitening System (Colgate)	Tam, 1999a
Quickstart (Den-Mat, USA)	Oltu and Gürgan, 2000
StarBrite (Interdent, USA)	Shethri *et al*., 2003

Opalescence Quick has been used predominantly as an in-office bleaching agent and has been clinically investigated regarding tooth sensitivity (Tam, 1999a). Opalescence Quick (35% carbamide peroxide) has been used to examine enamel surface roughness (Oltu and Gurgan, 2000; Worschech *et al*., 2003) and enamel surface changes or mineral loss (Zalkind *et al*., 1996; Cibrika *et al*., 1999) the latter two also form part of this study. In view of the many comparative studies using Opalescence Quick, it seemed appropriate to use this bleaching agent as a comparison to Ozicure Oxygen Activator.

2.1.3. Ozicure Oxygen Activator®

A new bleaching system has been introduced on the market called the Ozicure Oxygen Activator (O_3, RSA). The claim of the manufacturer is that this procedure is as efficient as conventional bleaching products with the added benefits of less or no tooth sensitivity or mucosal irritation during bleaching. In addition Ozicure Oxygen Activator is purported to be more environmental friendly than other methods. At present, there is no scientific evidence to support these claims and no published studies on the product. Therefore this is the first in vitro study to report on Ozicure Oxygen Activator.

This study will focus on the bleaching aspects of this product. Briefly the treatment consists of two phases of 30 minutes each (total treatment time of one hour). The first phase uses activated water, also called ozonated water, and ozone O_3 gas to irrigate the teeth. In the second phase, the teeth are bleached with Trèswhite strips containing nine percent hydrogen peroxide (Ultradent, USA). The tooth colour change and surface effects caused by the

Ozicure Oxygen Activator bleaching method will be investigated in chapter 4.

2.1.4. Variables associated with tooth colour change

Bleaching agents are usually applied to the teeth that are visible when one smiles (canine, incisor and premolar teeth). In the *in vitro* situation the researcher is restricted to teeth which are available for experimental use. Sulieman *et al*., (2005) and Spalding *et al*., (2003) were able to use canine, incisor and premolar teeth for their *in vitro* studies but had to section the teeth for reasons of availability and ease of use for further investigation. Other investigators have utilized molar teeth for their studies, mainly due to the availability of these teeth (da Costa and Mazur, 2007; Efeoglu *et al*., 2007; Worschech *et al*., 2003; Oltu and Gürgan, 2000; Flaitz and Hicks, 1996).

The present *in vitro* study had sufficient anterior teeth available to mimic the clinical situation by using intact, sound canine, incisor and premolar teeth, although it was not possible to have equal numbers of all teeth types.

2.1.4.1. Tooth type

Only one study has compared the response of different tooth types to bleaching. A study by Leonard *et al*., (1998) suggests that different tooth type's respond differently with bleaching agents. In his investigation canine teeth, responded better to certain bleaching solutions than incisor teeth. Unfortunately, there were insufficient premolars in the sample to draw conclusions for this tooth type. The present study will parallel the investigation

by Leonard *et al*., (1998) to establish whether different tooth types respond differently to a bleaching procedure, thereby providing more information in this area of bleaching.

2.1.4.2. Concentration of the bleaching agent

Clinically based scientific publications seem to agree that concentration of the bleaching agent has a positive effect on tooth colour change (Matis, Moosa, Cochran *et al*., 2000) and that the use of high concentration bleaching agents (30% to 35% hydrogen peroxide) produce instantaneous tooth lightening results (Kihn, 2007). This is confirmed in an *in vitro* study by Kielbassa *et al*., (2009).

2.1.4.3. Application frequency of the bleaching agent

Colour change following repeated tooth bleaching is less definitive. An *in vitro* study by Rosentiel (1991) has shown little benefit resulting from repeated bleaching beyond the tooth colour obtained from the first bleach treatment. This was confirmed *in vitro* by Kielbassa *et al*., (2009). However a clinical study by de Silva Gottardi (2006) indicates an average colour change of 2.1 - 3.7 units per appointment following a single and up to four in-office bleaching regimens to achieve patient satisfaction with tooth whitening.

2.1.4.4. Length of application time of the bleaching agent

In a study by Tam (1999a), patients wore their bleaching guards containing a low concentration bleaching agent (10% carbamide peroxide) during night times. Subjective tooth colour change was observed after an average of two and a half nights of tooth bleaching. Unfortunately the total bleaching hours was not mentioned in this study. Kielbassa *et al.,* (2009) have shown *in vitro* that the length of application time of low concentrations of home use bleaching products has no influence on the bleaching outcome.

2.1.4.5. Ability of the bleach to reach the stain

Dahl and Pallesen (2003) have stated that the success of a bleaching procedure is dependent on the concentration of the bleaching agent, the number of times the bleaching procedure is repeated and the ability of the bleaching agent to reach the discolouration or stain in the tooth. The latter provision is not covered in the literature. It is obvious that if the bleaching agent does not reach the stain, tooth lightening cannot occur. Numerous studies have indicated an increase in tooth permeability following bleaching procedures such as those for tooth enamel (Schiavoni, 2006) and dentine (Carrasco, 2007). However it cannot be assumed that increased tooth permeability following tooth bleaching implies that the bleach has been able to reach discoloured tooth structure.

2.1.4.6. Staining type and how best to treat it

While the category of stain may be readily apparent (section 1.2), there appear to be no clear guidelines on which bleaching procedure is best for the specific discolouration (Sulieman *et al*., 2003). Indeed there is a notable absence in the literature on which bleaching materials and methods are best used for different tooth stains on the different anterior teeth.

2.1.5. Summary of variables associated with tooth colour change

Data on the efficiency and duration of vital tooth bleaching are mostly related to clinical case presentation. Clinical indications are that most teeth are susceptible to bleaching. This section has examined six variables associated with tooth bleaching. Of the six variables, only the concentration of the bleaching agent appears to be confirmed in the literature as having a positive effect on tooth bleaching. The other variables: tooth type, application frequency, length of application time, stain type and the ability of the bleach to reach the stain have either ambiguous results or research is lacking in the field.

The lightening effect of tooth bleaching is expected to last one to three years. Bleached teeth discolour slowly over time and follow up bleaching treatments may be necessary (Haywood, 1996). This phenomenon is referred to as colour fall back or relapse and will be further discussed in the next section.

2.1.6. Tooth colour relapse

Tooth colour following bleaching is not maintained, inevitably staining continues and the teeth darken. What is not clear is whether bleaching alters tooth structure making the surface more susceptible to stain uptake. Stain uptake will contribute to tooth colour relapse.

What is also not clear is how many units tooth colour darkening constitutes tooth colour relapse. For the purpose of this study one unit will imply tooth colour relapse visually and digitally.

Timing of tooth colour assessment to determine the degree of relapse is critical in both short and long term. Kihn (2007) has pointed out that tooth colour can relapse to half a shade lighter due to the re-hydration of the teeth immediately following a bleaching treatment. A darkening of the teeth by two units on a VITA tooth shade guide (VITA, Germany), during the first six months after a bleaching treatment have been reported (Gerlach *et al*., 2002; Mokhlis *et al*., 2000).

Little information is available about tooth colour stability after tooth bleaching procedures. Clinically, colour relapses do occur after the completion of tooth bleaching treatments, but at present there is no predictor for the amount of tooth colour relapse that can occur. Most of the studies covered in the literature review, report a baseline and final colour reading taken at termination of treatment.

This *in vitro* study will investigate the continuous colour change occurring during the bleaching treatment and report on percentage of tooth colour relapse, using the chosen visual and digital tooth colour assessment data (in Chapter 4).

2.1.7. Tooth colour assessment

The broad background of tooth colour assessment has been covered in section 1.6.2 previously. For the purpose of this *in vitro* study, a spectrophotometer has been used for digital tooth colour assessment and a standard tooth shade guide for visual tooth colour assessment. Additionally this study will examine whether visual tooth colour assessment gives the same results as digital tooth colour assessment when the light environment is strictly controlled. Of all the studies surveyed only Kielbassa *et al.,* (2009) have described in detail the room light details during tooth colour determination. In this study the user instructions of the above colour assessment methods will be explained as well as the light conditions in which this occurred, (in Chapter 3).

2.1.7.1. Timing of tooth colour assessment

Tooth colour assessment in the studies reviewed is done at baseline prior to the bleaching treatment and directly after the bleaching treatment. Gerlach *et al*., (2002) argue that a twenty-four hour follow-up tooth colour assessment should be undertaken to compensate for any dehydration that may have resulted from the bleaching procedure. This is concurred by Kielbassa *et al*., (2009) who maintain that the colour reading taken 24 hours after bleaching is

the most stable. No previous studies have compared a one-hour and twenty-four hour colour follow-up tooth colour assessment to ascertain which is the more precise or indeed if it is necessary to do such a follow-up. Equally no studies have examined sequential change in tooth colour following repeated bleachings within a single study as is the case in this investigation.

2.2. Tooth surface changes

Tooth surface changes following bleaching can be grouped into two areas: one which examines changes in enamel surface roughness, the other regarding loss of tooth structure which can be established by a variety of tests.

In vitro studies have been mainly used to investigate tooth surface changes following bleaching with peroxides concentrations ranging from 5.3% to 38%. Joiner (2007) has reviewed 91 studies investigating changes in surface morphology and chemistry of enamel and dentine, as well as surface microhardness following bleaching. The majority (64) of these studies showed no substansive changes in enamel surface morphology for example Sulieman *et al*., (2004) and Lopes *et al*., (2002) following bleaching.

In contrast, Jaing *et al*., (2008) offered that high concentration bleaching agents should be used with caution, because they could have an adverse effect on the tooth enamel. Unfortunately, no detail has been given of these adverse effects. A few studies did find enamel changes consisting of enamel loss (Josey *et al*., 1996) and an increase in surface roughness or surface porosity (Worschech *et al*., 2003). An increase of pitting, exposure and loss of

underlying prism structure of enamel have also been found (Pirslin and Robinsson, 2004; Spalding *et al*., 2003; Flaitz and Hicks, 1996). Surface changes shown by the above *in vitro* studies did not replicate the *in vivo* setting or may be due to the low pH of the products used (Joiner, 2007). The above studies emphasize the uncertainty with regard to possible alteration to tooth structure that bleaching agents can cause.

The techniques used in this study to investigate tooth surface changes will be discussed and reviewed under the following headings: enamel morphology changes and surface roughness changes.

2.2.1. Enamel morphology changes

Scanning electron microscopy (SEM) is a well-established technique to investigate enamel morphology changes and calcium-phosphate levels after bleaching treatments (Barbour and Rees, 2004; Zalkind *et al*., 1996). A SEM uses a focused scanning beam of electrons to determine topography, morphology and composition of different samples (Sampson, 1996). Surface morphology is analyzed conveniently and described qualitatively by direct observation (Joiner, 2007).

Josey *et al*., (1996) suggested that mineral loss might have occurred, when bleached and unbleached enamel was investigated. Majeed *et al.,* (2008) reported a reduction in enamel microhardness after 112 hours of exposure to tooth bleaching agents, suggesting mineral loss. The use of carbamide peroxide on human enamel causes controversy when the SEM is used to

examine enamel surface alterations (Joiner, 2007). In addition chemical and microhardness changes to the enamel as well as changes to the underlying enamel prisms indicate surface loss.

Alteration to enamel morphology and chemical composition will be investigated in this *in vitro* study in conjunction with the SEM and associated energy dispersive x-ray spectroscopy, EDX, to try to establish if any surfaces changes have occurred after the bleaching treatments using Ca:P ratios as an indicator.

2.2.2. Surface roughness changes

Although the SEM has been used to assess surface roughness change following tooth bleaching, the inconclusive nature of the results may be due to technique limitations (Appendix F). A more conservative method to examine enamel surface roughness change following tooth bleaching may be the Atomic Force Microscope (AFM).

There have been two methods used to determine enamel surface roughness in previous tooth bleaching studies: atomic force microscopy and profilometry.

Results with the profilometer method have been contradictory. Worchech *et al*., (2003) found that the bleaching agent Opalescence Quick did not alter enamel surface roughness when sound enamel fragments were bleached for one hour for four weeks with seven day intervals. Titley *et al*., (1998) on the other hand found an increase in enamel surface roughness when 35% hydrogen peroxide was used.

A more sensitive method of establishing surface roughness is using the atomic force microscope. The AFM determines surface roughness of small areas of samples. This microscope uses a tip to run over or pass close to the surface of the sample and measures the deflection of a cantilever by the sample to detect the local height of the sample and establish its topography (Baselt, 1993). The advantages of this method are minimal preparation and the ability to characterize the surface in the X, Y, and Z directions. The examination conditions are close to the natural conditions and the resolution can be down to the atomic level. Only one previous study has employed the AFM to examine surface change following bleaching. Hegedüs *et al*., (1999) report that following bleaching with 30% hydrogen peroxide, the surface morphology of treated enamel changed compared to untreated enamel. The bleached enamel surface was reported as being relatively smooth with grooves which had a rougher appearance when compared with untreated enamel. This they speculated could be due to organic material loss.

The paucity of studies on surface roughness and bleaching makes it unclear what role surface roughness plays in tooth bleaching and to what extent increased surface roughness may affect the tooth over time. Indeed whether surface roughness and enamel loss plays a role in bleach penetration is an open question which begs an answer.

2.3. Study controls

Scientific studies use controls to test variables within a study. The control is almost identical to the actual study and differs in the fact that only the factor

being tested is left out of the control. This results in a controlled setting to test the factor in question and all other variables are excluded. Two study controls for this investigation required clarification from published literature. The one involved suitable comparison of tooth surfaces between bleached and unbleached surfaces, the other a suitable control for the bleaching treatment itself. The next sections will deal with each of these in turn.

2.3.1. Tooth surface control for bleaching

There are different ways to formulate a control to compare the surface effects of bleaching. One way would be to use two different teeth, the one tooth being treated with the bleaching agent and the other being the control. The problem with this method is that normal surface roughness, and Ca:P ratios differs between teeth, possibly compromising results. This can be overcome by enlarging the study sample. However this could lead to prohibitively unmanageable numbers considering the time consuming nature of the investigation.

Another method is to use the same tooth as its own control by protecting part of the surface from the bleaching agent during bleaching. Nail varnish has been used in scientific studies to cover and protect tooth surfaces against bleaching agents (Gökay *et al*., 2008). The tooth area to be bleached is left uncovered and all the surrounding tooth surfaces are covered with nail varnish (Pretty *et al*., 2001). The areas covered with nail varnish serve as the control of the individual teeth. Bleaching procedures only commence when the nail varnish has set (Schemehorn *et al*., 2004).

The nail varnish method was selected to compare pre and post bleaching surfaces. A pilot study was undertaken to refine the technique using two different coloured nail varnishes. Red and clear varnish were tested for suitability for this study (Appendix B).

2.3.2. Bleaching control

In this *in vitro* study, distilled water was used as a control for the two bleaching agents: Opalescence Quick (Ultradent, USA) and Ozicure Oxygen Activator (O_3, RSA). Another study has used tap water as a control (Kielbassa et al., 2009) but this was considered unsuitable in the South African context. The control ensured the precise measurement of the bleaching action of the two bleaching agents under investigation.

2.4. Aim and research hypotheses

The exhaustive review of literature around tooth bleaching, tooth colour changes and the effects of the procedures on the enamel surface structure have revealed a number of gaps in the knowledge. This enabled a number of hypotheses to be formulated that would be tested in the experimental design.

The aim of the study was to compare the two in-office bleaching agents Ozicure Oxygen Activator (O_3, RSA) with Opalescence Quick (35% carbamide peroxide) (Ultradent, USA).

The hypotheses which were to be tested are as follows:

1. Tooth colour change with Ozicure Oxygen Activator is superior and more stable to that obtained by Opalescence Quick.

2. The three tooth types (canine, incisor and premolar) respond differently to bleaching.

3. Teeth will become progressively lighter with ongoing bleaching.

4. Tooth colour assessment methods will reflect a similar trend of tooth bleaching outcomes between the two bleaching agents.

5. The bleaching process does not cause any change to the enamel tooth surface in terms of surface roughness or tooth surface loss (as measured by Ca:P ratios) .

2.5. Objectives of the study

The objectives of this *in vitro* study were formulated as follows to test the hypotheses.

1. Tooth colour obtained from Ozicure Oxygen Activator bleach will be compared to that of the gold standard Opalescence Quick and a control (distilled water).

Three tooth types (canine, incisor and premolar) will be bleached three times with tooth colour assessments done at three intervals (baseline, one hour and 24 hours after each bleach) using visual and digital tooth colour assessment methods.

2. Determine if colour relapse occurs between bleaching treatments and the overall colour stability between readings.

A comparison will be made between the 24 hour tooth colour following the first tooth bleaching treatment and the baseline tooth colour before the second treatment commences. The 24-hour tooth colour (first bleach) will also be compared to the 24-hour tooth colour (third and final bleach).

3. Comparison between the bleaching obtained when measuring teeth by visual and digital colour assessment.

Visual and digital tooth colour assessment methods will be compared to see if the same trend in bleaching results is achieved. Both methods will be used on all the teeth throughout the study and the lightening effect will then be compared.

4. Ascertain whether the bleaching process causes any tooth enamel surface changes. This will be done in two ways:

a. A small area of the middle third of the palatal/ lingual surface of the tooth crown will be covered with two layers of nail varnish to protect it from the

bleaching agent/control. Transversely sectioned tooth crowns with the least and the most colour change in each group following experimentation will be examined using SEM and EDX. The Ca:P ratios of the nail varnish covered and exposed tooth enamel surfaces will be compared for a difference in these ratios, which might indicate tooth mineral loss in the exposed tooth surfaces. A change in surface levels of these two areas would also be an indication of tooth surface loss.

b. AFM will be used to determine surface roughness of the three experimental treatments.

5. Comparison of the two colour assessment methods in terms of ease of use.

The two methods will be compared with regard to their ease of use and how much time each method utilizes to produce colour assessment. The cost difference between the two methods will be reported.

Chapter 3 Materials and methods

The methodology was refined through a series of pilot studies which are reported in the different Appendices at the end of this research report. The separation of pilot studies from the final methodology has been done to prevent readers of the report from loosing the course of the method. The methodology as presented in this chapter is the final procedure undertaken for this study.

3.1. Ethics clearance

Permission to use human extracted teeth in this study was obtained through the HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL) at the University of the Witwatersrand, Johannesburg, ethics clearance certificate (M050760). A copy of this certificate can be found in Appendix A.

3.2. Tooth selection

Teeth used in the study were obtained from those accumulated by the Dental Research Institute, University of the Witwatersrand. Unfortunately stain type, which is heavily reliant on clinical history, could not be determined with any accuracy and could not form a part of this study.

A pool of two hundred and fifty teeth was initially selected for the study according to the following criteria: incisor teeth, canine teeth, premolar teeth with a darker tooth colour than 1M2. The external debris and stains on the teeth were removed with a Cavit-Jet (Cavitor Dentsply, USA) scaler. A

polishing cup [KerrHawe Optishine™; Batch number 10/Art. No.2514 (Switzerland)] and polishing paste [Nupro prophylaxis paste with fluoride (Dentsply, USA)] were used to polish the tooth surface for one minute. The teeth were re-examined and only those with no caries, no surface defects and within the colour ranges 1M2 to 5M3 were included in the study to give 136 teeth (Figure 3.1). The colour of the teeth was established visually with a VITAPAN 3D MASTER TOOTH GUIDE (VITA, Germany) and fell within the range of the teeth most commonly treated with bleaching. The teeth were stored in individual bottles containing a few thymol crystals (Merck, Germany) at six degrees Celsius following collection (Figure 3.1).

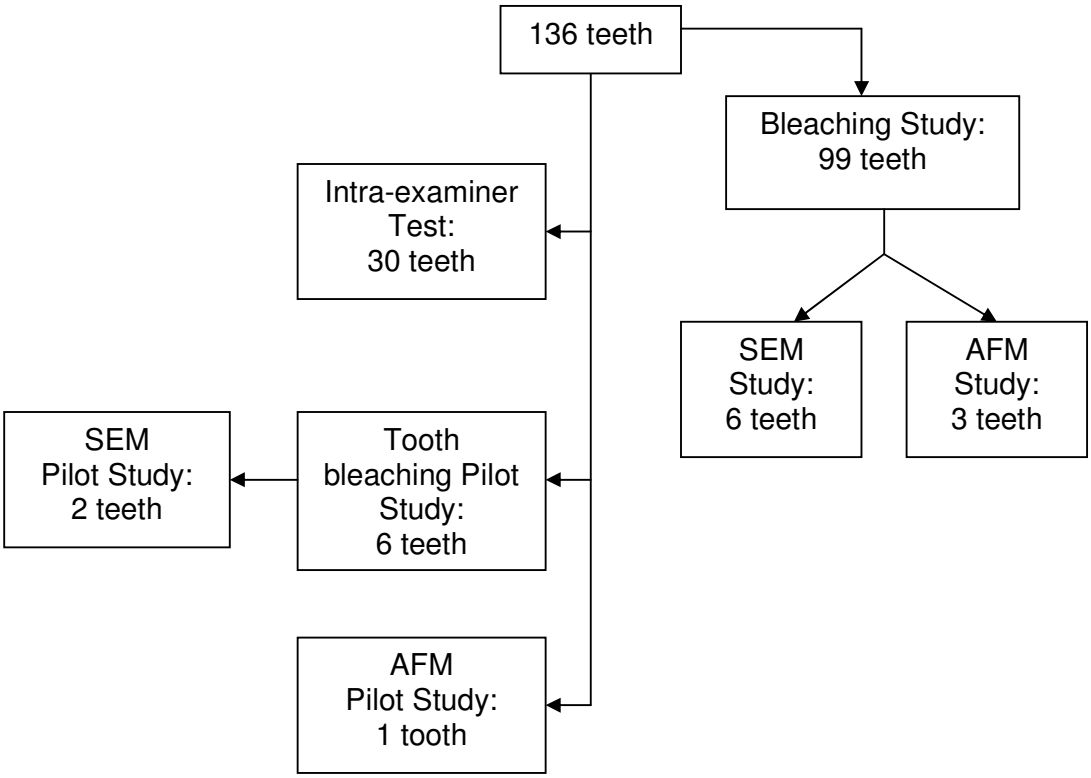


Figure 3.1. Flow diagram indicating how the 136 teeth were used in the study.

The intra-examiner test will be covered in section 3.7.1.

The tooth bleaching pilot study can be found in Appendix E.

The AFM pilot study will be covered in Appendix G.

The bleaching study will be discussed in Section 3.3.

The SEM pilot study will be covered in Appendix F.



Figure 3.2. All the teeth in the study were stored in individual labelled bottles containing 1% thymol (MERCK, Germany).

Thereafter teeth were randomly divided, by tooth type and then subdivided into three groups according to the experimental treatment. Each tooth was coded and placed into the corresponding individually numbered bottle.

3.3. Bleaching study

The 99 teeth intended for the bleaching study were divided into three experimental groups of 33 teeth each: Ozicure, Opalescence Quick and the Control group. The 33 teeth were further divided into three replicates of 11 teeth apiece consisting of four incisors, two canines and five premolars. The arrangement of experimental groups is illustrated in Table 3.1. Before each bleach application the teeth were removed from the bottles and the individual teeth placed in the sockets of the custom made acrylic base plate (Appendix D) specific for the replicate and experimental group.

The precise manufacture of each base plate ensured that each tooth had its

own specific socket and there could be no accidental mixing of teeth. Each tooth in the three groups received three bleaching treatments on consecutive days.

Table 3.1. Arrangement of experimental groups.

Experimental Groups		
Control group	Opalescence Quick group	Ozicure group
n=33	n=33	n=33
Replicate: 1 n= 11 5 premolars 4 incisors 2 canines	Replicate: 1 n=11 5 premolars 4 incisors 2 canines	Replicate: 1 n=11 5 premolars 4 incisors 2 canines
Replicate: 2 n=11 5 premolars 4 incisors 2 canines	Replicate: 2 n=11 5 premolars 4 incisors 2 canines	Replicate: 2 n=11 5 premolars 4 incisors 2 canines
Replicate: 3 n=11 5 premolars 4 incisors 2 canines	Replicate: 3 n=11 5 premolars 4 incisors 2 canines	Replicate: 3 n=11 5 premolars 4 incisors 2 canines

3.3.1. Bleaching procedures

There were three experimental groups namely: Ozicure, Opalescence Quick and the Control group. The teeth in each group underwent three cycles of one hour treatments each on three consecutive days.

3.3.1.1. Ozicure Oxygen Activator

The Ozicure Oxygen Activator bleaching procedure consisted of two phases. These were carried out strictly according to the manufacturer's instructions.

3.3.1.1.1. First phase

During this phase, the teeth were wetted with activated water that goes with the instrument, placed into the base model and covered with the bleaching guard.

The Ozicure Oxygen Activator was attached via a small diameter pipe to the bleaching guard inlet. This pipe transfers the ozone gas (3l/min) from the Ozicure Oxygen Activator into the bleaching guard. The ozone gas reacts with the teeth that are wetted with the activated water and bleaches the teeth. The ozone gas is extracted by the dental suction pump at a rate of 5l/min (Figure 3.3).

After 15 minutes, the bleaching guard was removed and the teeth were again wetted with activated water. The bleaching guard was immediately repositioned over the teeth. This procedure took 30 minutes. Figure 3.3 and 3.4a-h illustrate the first phase of the Ozicure Oxygen Activator bleaching procedure.

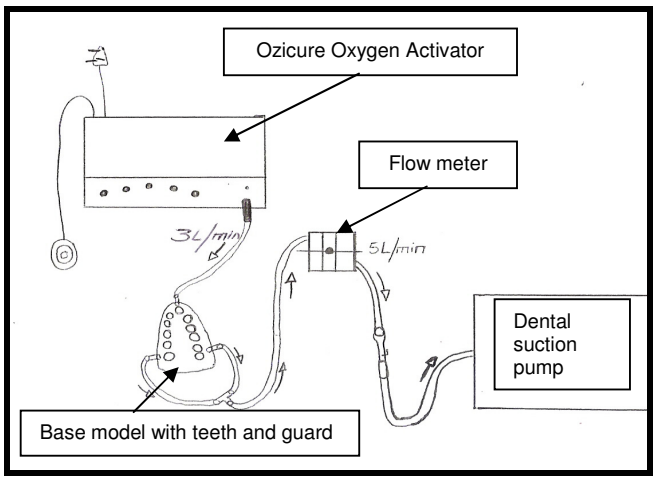


Figure 3.3. A diagrammatic illustration of the bleaching procedure using the Ozicure Oxygen Activator.

3.3.1.1.2. Second phase

The second phase followed directly after the first phase and involved the bleaching of the teeth with Trèswhite (Ultradent, USA) strips. Trèswhite (Ultradent, USA) strips contain nine percent hydrogen peroxide. These strips were sculpted over the teeth (Figure 3.5 a-b).

The Trèswhite (Ultradent, USA) strips were removed after 15 minutes and the teeth were moistened with distilled water. The strips were repositioned on the teeth and left for another 15 minutes. The total time of the procedure was 30 minutes. After the procedure, the teeth were rinsed with distilled water and cleaned with paper towel. The colour of each tooth was determined immediately following the treatment and 24 hours later.



Figure 3.4a. Teeth in the Ozicure group was placed in the base model and moistened with activated water.



Figure 3.4b. The bleaching guard was placed in position over the teeth and the base model, providing a good tight fit and an optimum seal.

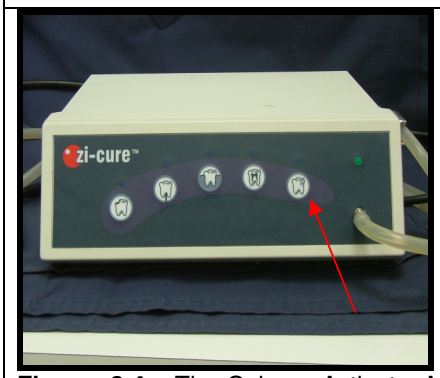


Figure 3.4c. The Ozicure Activator Machine was switched on and the bleaching option was chosen (red arrow).



Figure 3.4d. The Ozicure Activator Machine was attached to the bleaching guard and the process was started with the foot pedal (red arrow).

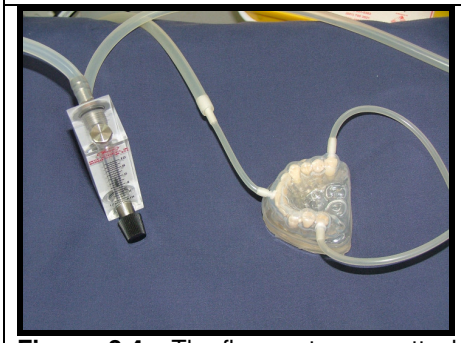


Figure 3.4e. The flow meter was attached to the bleaching guard and the suction pump. The flow meter regulated the removal of the ozone gas from the guard that covered the teeth in the base model. The ozone gas was extracted at a rate of 5l/min.



Figure 3.4f. Ozone gas was extracted from the guard at a rate of 5l/min.

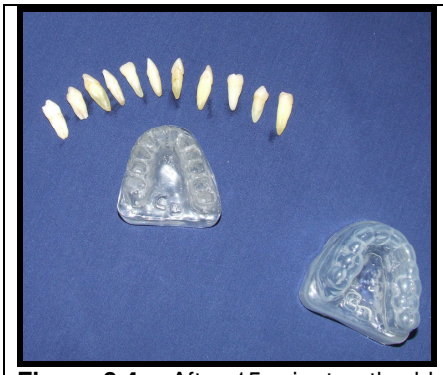


Figure 3.4g. After 15 minutes the bleaching guard was removed and the teeth were moistened with the activated water. The bleaching guard was placed in position over the teeth and the procedure continued for 30 minutes.

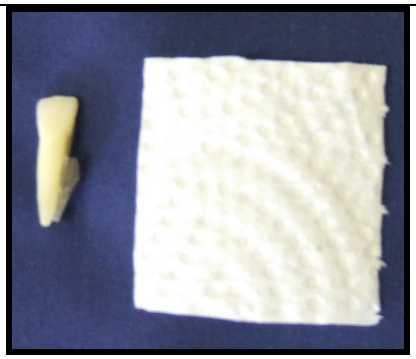


Figure 3.4h. After the 30 minutes cycle, the Ozicure Activator Machine automatically switches off. The teeth were dried with paper towel and the second phase followed.

Figure 3.4a-h. First phase of the Ozicure Oxygen Activator bleaching procedure.



Figure 3.5a. The transport tray for the Trèswhite (Ultradent, USA) strips.



Figure 3.5b. Trèswhite (Ultradent, USA) strips were adapted over the teeth.

Figure 3.5. Second phase of the Ozicure Oxygen Activator bleaching procedure.

3.3.1.2. Opalescence Quick

Opalescence Quick (Ultradent, USA), a gel containing 35% carbamide peroxide was used in the Opalescence Quick group. The teeth were placed in position on the model (Figure 3.6a). The gel was placed into the bleaching guard with the syringe (Figure 3.6b and 3.6c). The bleaching guard containing the bleaching agent was placed in position over the teeth. The bleaching guard ensured full contact of the bleaching agent on the coronal aspect of the teeth (Figure 3.6d).

The total bleaching time was one hour with 15 minute intervals. During the intervals, the bleaching guard was removed and the teeth were wetted with distilled water using a spray bottle. New bleaching agent was added to the bleaching guard and agitated. Tooth colour was determined immediately following the treatment and 24 hours later.



Figure 3.6a. Teeth were placed in position in the mould.

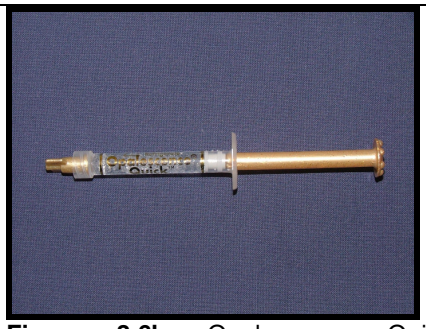


Figure 3.6b. Opalescence Quick gel, containing 35% carbamide peroxide (Ultradent, USA).

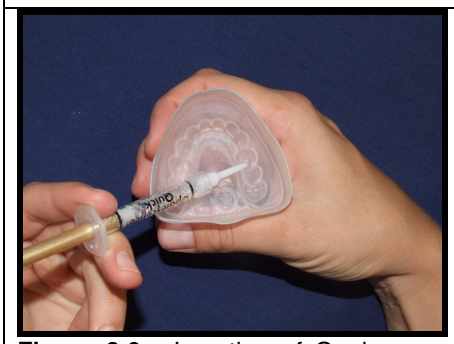


Figure 3.6c. Insertion of Opalescence Quick into the bleaching guard.



Figure 3.6d. The Opalescence Quick gel was placed into the bleaching guard and the bleaching guard was placed over the teeth.

Figure 3.6a-d. The Opalescence Quick bleaching procedure.

3.3.1.3. Control group

The treatment of the teeth in the control group (Figure 3.7a) was exactly the same as the Opalescence group with 15 minute intervals. After the initial 15 minute treatment was completed, the teeth were rinsed with distilled water from a spray bottle (Figure 3.7b). The guard was replaced and the treatment restarted (Figure 3.7c). The only difference in the treatment was that the active bleaching ingredient was replaced with distilled water. The tooth colour assessments were determined immediately following the treatment and 24 hours later.



Figure 3.7a. Teeth were placed in position on the control base model.



Figure 3.7b. The guard was removed and the teeth were rinsed with distilled water.



Figure 3.7c. The guard was placed over the teeth on the base model.

Figure 3.7a-c. The control group bleaching procedure.

3.4. Tooth colour assessment

Two methods were used for tooth colour assessment: visually with the VITAPAN 3D MASTER TOOTH GUIDE (VITA, Germany) and digitally with the VITA Easyshade spectrophotometer (VITA, Germany). Tooth colour assessments were taken at baseline prior to bleaching, one hour and 24 hours after the bleaching procedure for each of the three bleaching procedures.

3.4.1. Visual tooth colour assessment

The VITAPAN 3D MASTER TOOTH GUIDE (VITA, Germany) has a maximum of 29 tooth colours from which to choose (Figure 3.8a) and works by the following three simple steps:

1. The value or lightness of the tooth colour is established.

The value indicates the lightness of the tooth colour ranging from pure black to white. The upper row of shade samples (1M1, 2M1, 3M1, 4M1, 5M1) are held against the tooth (Figure 3.8b) and the value of the tooth colour is established, for example 2M1.

2. The second step is to use the vertical tabs to determine the chroma or the intensity or saturation of the tooth colour, for example 2M2.

3. The third step is to determine the hue of the tooth colour using the horizontal tabs. The shade samples 2L and 2R are now used to determine if the tooth colour is more yellow (2L) or more red (2R).



Figure 3.8a. VITAPAN 3D MASTER TOOTH GUIDE (VITA, Germany).

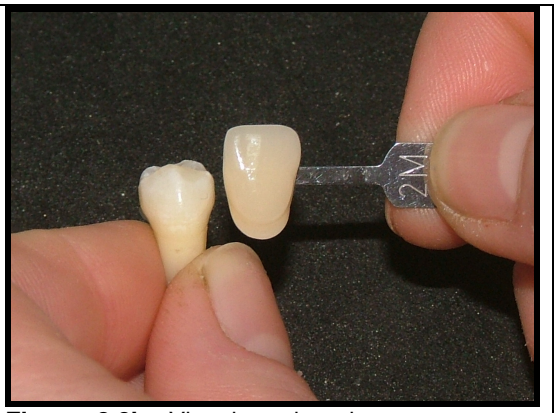


Figure 3.8b. Visual tooth colour assessment using the VITAPAN 3D MASTER TOOTH GUIDE. The shade tab is held against the tooth to determine an accurate tooth colour.

Figure 3.8. Visual tooth colour assessment.

For the statistical evaluation of the results the shade tabs (Figure 3.8a), which record shades alpha-numerically, were numbered consecutively from the lightest (0M3) to the darkest (5M3) colour. This gave a ranked numerical series from one to 29. These ranked numbers were used for the statistical and descriptive analysis of the data.

3.4.2. Digital tooth colour assessment

The VITA Easyshade spectrophotometer (VITA, Germany) has a maximum of 81 colours from which to choose (Figure 3.9a).

Prior to each use the probe tip was placed flush and perpendicular to the calibrating block for automatic colour calibration as required by the manufacturer. For each tooth colour assessment the setting “SINGLE TOOTH” was selected with the “AVERAGE” setting “OFF” used from the menu bar. The spectrophotometer produced a single tooth colour reading for the selected

tooth. The probe tip was placed flush on to the tooth and perpendicular to the tooth surface and an assessment was done (Figure 3.9b). Three consecutive tooth colour assessments are needed to confirm tooth colour as per the manufacturer's instructions.

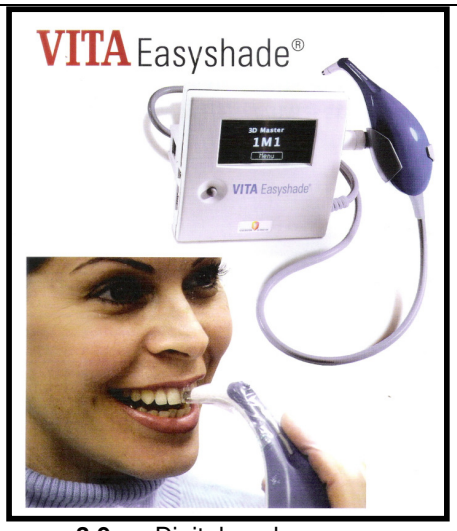


Figure 3.9a. Digital colour assessment using the VITA Easyshade (VITA, Germany).



Figure 3.9b. Digital tooth colour assessment using the VITA Easyshade (VITA, Germany). The instrument is placed flush on the tooth and perpendicular to the tooth surface. Three corresponding tooth colours are needed for a positive tooth colour match.

Figure 3.9. Digital tooth colour assessment using the VITA Easyshade.

As was the case with visual colour assessment, each of the 81 tooth shades which have alpha-numeric shade values was rank numbered from the lightest (0M3) to the darkest (5M3). These numbers ranked from one to 81 were used in the statistical and descriptive evaluation.

Tooth colour shades were captured on a Microsoft Office Excel spreadsheet. These shades were converted to the corresponding numerical value. It is these numerical values which were analysed using SAS (SAS for Windows Version 9.1, SAS Institute Inc., Cary, NC: USA) as well as being used in the statistical

and descriptive analysis.

3.4.3. External light conditions during colour readings

The study took place in the same dental surgery. In the surgery, the same position was always used during tooth colour assessment. The walls of the surgery were painted a light grey matt finish. Day light globes were the only light source used. External light sources were blocked out by closing the blinds in front of all the windows and the surgery door was kept closed. A black matt background was used during tooth colour assessment for both methods.

3.4.4. Summary of sequence of tooth colour readings

In summary the sequence of colour readings was as follows:

Baseline colour reading at the start of the experiment.

First bleach treatment followed by a one hour and 24 hour colour reading.

Baseline colour reading before second bleach, second bleach treatment followed by a one hour and 24 hour colour reading.

Baseline colour reading before third bleach, third bleach treatment followed by a one hour and 24 hour colour reading.

Thus each tooth had nine sequential colour shade readings. In total 891 tooth colour readings were made in this study. This data was used to assess the influence of the bleaching agents on tooth colour change. Tooth colour relapse was deemed to occur with the colour of the tooth darker than the previous measured colour by one unit.

3.5. Statistical design and analysis

A statistician was consulted for the appropriate study design. On his advice a randomized block design was used to examine and analyse tooth colour change. Statistical analysis was done using the General Linear Models (GLM) test for Analysis of Variance for a Fractional Design set at a significance of $P<0.05$. The bleaching methods, number of tooth bleaches and tooth type were the independent variables of the study. The tooth colour was the dependent variable. The data was analysed in selected subsets to further explore the interactions within the study.

3.6. Descriptive statistics

Descriptive statistics using the rank number to obtain mean values, standard deviations maximum and minimum readings were used to further investigate the dynamics of the study. For instance: first and final tooth colour mean values and standard deviations were compared to create an argument for the "best" bleaching system (Opalescence Quick or Ozicure Oxygen Activator). The three tooth types were similarly compared to establish which tooth type react the best to the bleach treatment and so on. This will be further elaborated upon in the results.

3.7. Blinding and validation of study procedures

The researcher was blinded during the entire study to the previous tooth colour readings made both visually and digitally.

3.7.1. Intra-examiner test

The purpose of the intra-examiner test was to ensure consistent colour assessment using the visual method of tooth colour reading.

Thirty teeth were randomly selected for the test and stored in 30 individual labelled bottles. Tooth colour was visually assessed four times on four consecutive days. The test was concluded blind to the previous readings. The test was performed in the same surgery under identical light conditions against which the teeth were read using a black matt background. A Cochran-Mantel-Haenszel test was performed on the raw data and P= 0.1841 established. There was no significant difference between the visual assessments thus indicating that tooth colour was consistently read.

3.8. Tooth surface effects

Microscopy was used to determine if any surface roughness and mineral loss were caused by the bleaching procedures. A standard method was used to investigate possible tooth mineral loss using energy dispersive x-ray spectroscopy (EDX) on the SEM. The Atomic Force Microscope was the preferred method to determine the tooth surface roughness.

3.8.1. Mineral loss - scanning electron microscope procedure

Following the completion of the bleaching procedure two teeth were selected from each experimental group, (Ozicure, Control and Opalescence). The two teeth were selected according to the following criteria: the tooth with least colour change after treatment and the tooth with most colour change after treatment (Table 3.2). Thus six teeth were used in this part of the study.

Table 3.2. Samples used for SEM investigation.

Sample	Experimental Group	Tooth type	Tooth number	Colour change	Final tooth colour
1	Opalescence 1	Premolar	11	Least	2M2
2	Ozicure 3	Premolar	1	Least	3M3
3	Control 2	Premolar	10	Least	2M3
4	Control 1	Premolar	2	Most	3R2.5
5	Opalescence 2	Incisor	7	Most	2M1
6	Ozicure 3	Premolar	2	Most	2M2

The teeth were left to dry on a paper towel for 24 hours and embedded using a double embedding process. Figure 3.10a-h illustrates the materials and equipment used during the embedding. Curing of the resin took place overnight at 40ºC in an incubator.



Figure 3.10a. Araldite M Resin and HY956 hardener (PLASTOMAX CC/BK). The resin based material was used for the embedding. The resin was mixed with a hardener in a ratio of five to one according to weight.



Figure 3.10b. Sartorius scale used for weighing the resin and hardener.

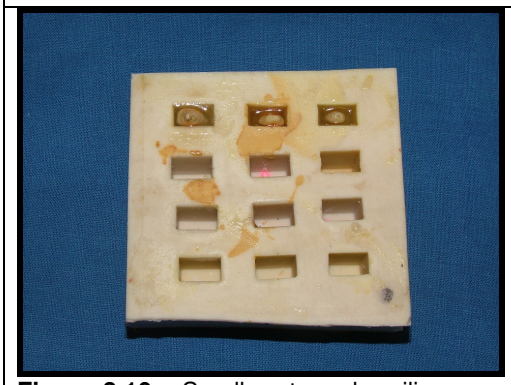


Figure 3.10c. Small rectangular silicon mould used for the first embedding.

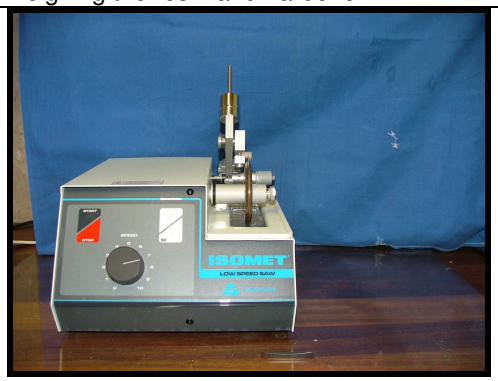


Figure3 .10d. The ISOMET (BUEHLER, Germany) is a low speed, water cooled, diamond tipped saw.



Figure 3.10e. The embedded tooth was sectioned at a speed setting of eight. [Diamond wafering blade, series 15HC diamond no. 11-4244 (BUEHLER®, USA)].

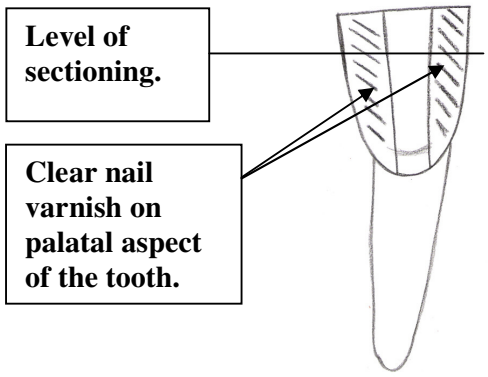


Figure 3.10f. Schematic illustration of the palatal aspect of an incisor tooth covered by clear nail varnish and the level of sectioning.



Figure 3.10g. Second embedding in round moulds.

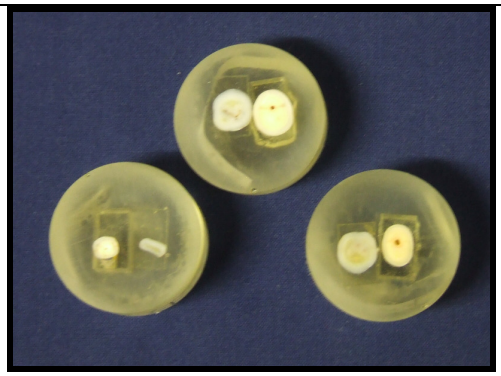


Figure 3.10h. Tooth specimens embedded and polished.

Figure 3.10a-h. Double embedding procedures for (SEM) viewing and polishing.

The tooth which was transversely sectioned midway through the crown as illustrated in Fig 3.10e and f, was left to dry for 24 hours. A round mould with the inner surface lightly coated with petroleum jelly (Vaseline) was used for the second embedding (Figure 3.10g). An identifying label was placed with the number facing outward. The two sectioned parts of the tooth were then embedded together with the areas of interest facing down using the same resin mix and curing regimen as before.

3.8.1.1. Polishing of SEM samples

The double-embedded sectioned tooth specimens were hand polished using an IMPTECH 20 DVT GRINDER POLISHER. The polisher was set at a speed of 300rpm and a continuous stream of water was used to lubricate the polishing disc and sample block surface. The rim of the specimen was bevelled at the rear side and the sample side with an 80 grit grinding paper. This ensured that the polishing paste would be drawn under the specimen surface during the final polishing. The grinding papers were used in decreasing polishing grit size: 80; 180; 320; 400. The specimen block was rotated 90° for each successive polishing on a grinding paper.

The researcher washed his hands continuously during the polishing of the specimens to prevent contamination of the polishing surface. An ultrasonic bath was filled with water to a level of about three to five centimetres high. A glass beaker containing 100 millilitres of alcohol was placed in the ultrasonic bath. The specimens were held face down in this glass beaker with artery forceps for 15 seconds after every polishing stage to remove polishing debris. Paper towel was used to dry the samples before viewing with the light microscope.

Incident light was angled to examine the sample surface. This showed the profile of the polished surface in order to determine the optimum polishing depth.

The samples were given a final polish with the 3.0µm and 1.0µm grade diamond discs to remove any scratches and to produce a smooth surface. DP – Lubricant Red [(Lubricating liquid or diamond polishing, 1L), Struers A/S, Denmark] was use as lubricant with the 3.0µm and 1.0µm polished discs instead of water.

3.8.1.2. Mounting and coating of SEM samples

The SEM samples were mounted on a stub using Dag® 580 [(Colloidal graphite in denatured alcohol), Acheson Industries (Ltd) P.O. Box 9679 ZG SCHEEMDA, The Nederlands]. The samples were carbon coated in an Edwards Vacuum Coating Unit, model E12E (Edwards Vacuum Ltd, Crawley, UK) for conducting purposes (Figure 3.11). Prior to viewing, the nail varnished area was marked to ensure correct location of the exposed and unexposed bleached tooth surface.

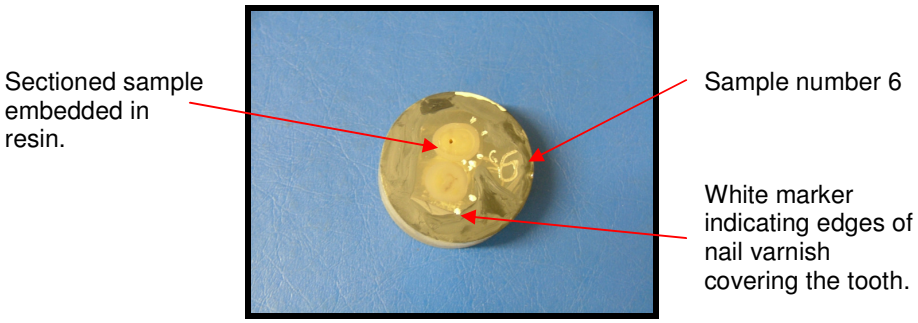


Figure 3.11. SEM sample coated with carbon.

3.8.1.3. Viewing of SEM samples

The polished, coated tooth specimens were viewed in a LEO 1525 FESEM (Field Emission Scanning Electron Microscope, Carl Zeiss NTS GmbH, Oberkocken, Germany). Surface enamel elemental analysis was undertaken in two areas of the polished specimen using an Oxford INCA energy dispersive x-ray analyzer (Oxford Instruments, High Wycombe, UK). Analysis conditions were 20kV utilizing a live counting time of 100 seconds. Sample analyzed areas were typically 100µm by 10µm (Figure 3.12). The analyzed areas were in the region immediately below the exposed tooth surface, as indicated by the green star in Figure 3.13. The other analyzed areas were immediately below the varnish-covered areas (control) indicated by the green star in Figure 3.14. The x-rays generated originated from a depth of up to about 3.0 µm inside the enamel (Anderson, 1973).

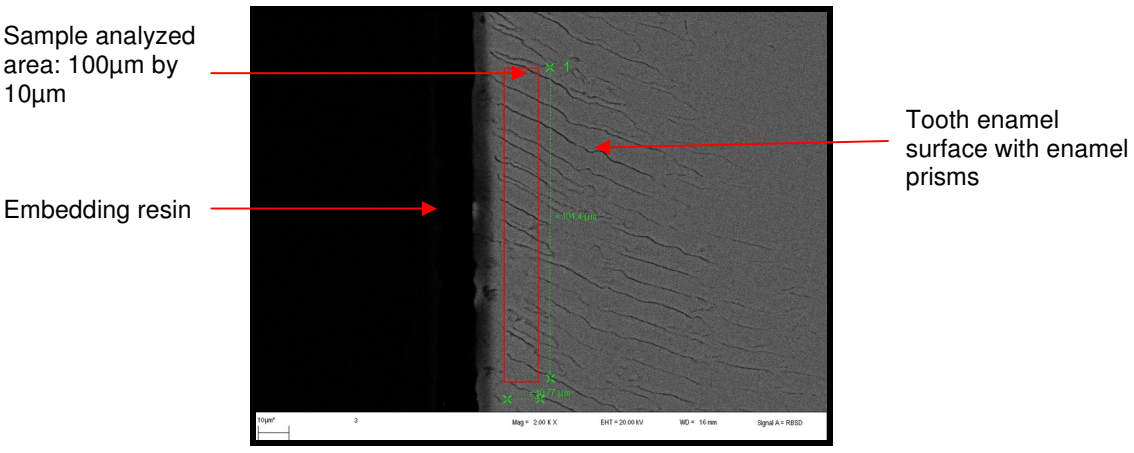


Figure 3.12. Typical analyzed area was approximately 100µm by 10µm represented by the red rectangle.

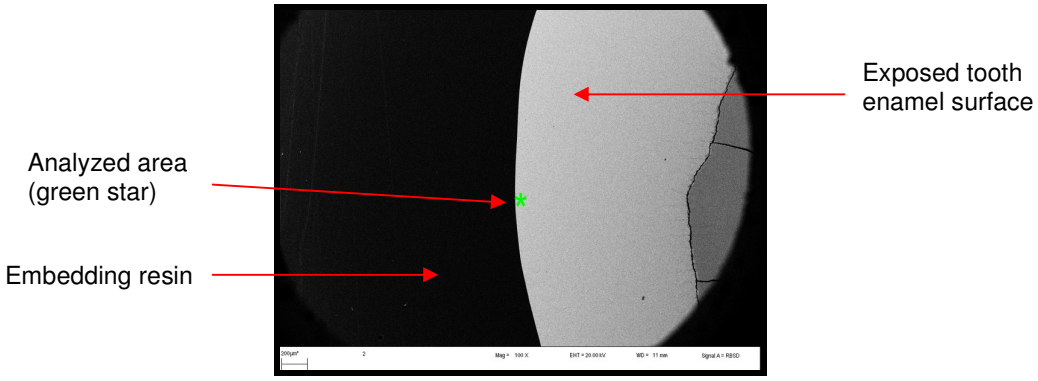


Figure 3.13. The green star represents the analyzed area on the exposed tooth enamel surface.

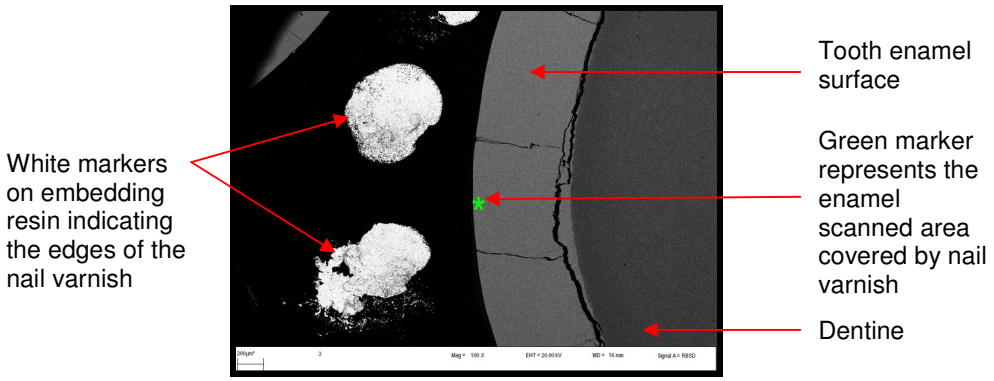


Figure 3.14. The green star represents the analyzed area on the nail varnish covered tooth enamel surface.

Six teeth were scanned (Table 3.2) and the Ca:P - ratio data was captured, saved and analyzed using a Microsoft Excel spreadsheet. Two scans were made in the enamel areas covered by nail varnish and two scans in the exposed enamel areas.

Micrographs of the enamel were taken to determine if any morphological differences were apparent between bleached and protected areas.

3.8.2. Surface roughness - atomic force microscope procedure

The intention of the *in vitro* study was to establish surface roughness for the tooth with the least colour change and the tooth with the greatest colour change in each experimental group. Unfortunately the pilot studies undertaken for this aspect of the study did not indicate problems which emerged when the experimental teeth were viewed. These were mainly due to constraints caused by the curvature of the teeth themselves. Thus only one tooth was scanned in each group with a variable number of scans.

3.8.2.1. Cleaning and mounting of AFM tooth samples

After bleaching the selected teeth were allowed to dry. Each tooth was cleaned with a cotton bud, soaked in ethanol (CAS 64175). The teeth were dried with an Easy Duster (SPI Supplies), Wert Chester, USA with batch number *+H6Ø6Ø76Ø5ABLO*. Each tooth was mounted on a round steel disc, so that the surface on the tooth was mounted parallel to the disc surface. (The three mounting methods explored can be found in Appendix G).

3.8.2.2. Viewing of the AFM samples

The teeth were positioned with Pratley Quickset Clear (Pratley®) on the magnetic sample holder in the AFM. Scanning was restricted in the X and Y-axis to a distance of ten micrometres due to the curvature of the teeth. Scans from three different regions were obtained from each tooth. The factors that can be determined by AFM are (a) Maximum height features, (b) Median height features given by the maximum vertical value (the maximum number of points with a given height). The di PROSCAN SOFTWARE VERSION 2.1 IMAGE PROCESSING was used to capture all the data.

Once the images were acquired, they were processed in three steps: The first step was to flatten the images; this is done to remove non-linearity from the images due to motion of the scanner. In the second step, some images were deglitched in order to remove artefacts i.e. showing up as noise in the images. The deglitching works by equalizing a linear function of the data within the selected region. In the third step, the entire image or region of interest was selected to measure the roughness of the feature of interest excluding regions of noise.

The images were saved to the hard drive in the directory c:\spmdata. IP 2.1 software was used for analyzing the images. Data acquisition software (Linear analysis and region analysis) was used to measure the actual values of the image and collect statistics. The data was stored as a HDF file format (hierarchical data format). The images were edited (deglitched) in the 2D window to emphasize the areas of interest. The images were analyzed using

the FFT analysis to derive the 2D power spectrum of the image and the notch filter was used to remove peaks due to noise. A height histogram was plotted from the distribution of heights along the selected height profile.

Technical problems and further uniformity constraints resulted in only three teeth being scanned for surface roughness with varying numbers of usable scans. The incisor tooth with the least tooth colour change was used in the Opalescence Quick group and 10 scans were utilized. In the control a premolar with the least tooth colour change was used and produced 10 scans. Unfortunately for the Ozicure Oxygen Activator group only seven useable scans were taken from the canine with the least tooth colour change due to the curvature of the crown surface.

Chapter 4 Results

4.1. General

For the purpose of this study, visual tooth colour assessment will refer to tooth colour assessment captured with the VITAPAN 3D MASTER TOOTH GUIDE (VITA, Germany). Digital tooth colour assessment will refer to the tooth colour assessment captured with the VITA Easyshade (VITA, Germany).

Both Opalescence Quick and Ozicure Oxygen Activator bleaching treatments caused tooth lightening. During the treatment phase, the bleaching guards effectively brought the bleaching agents in contact with the teeth. This was confirmed in the case of Opalescence Quick by the gel adhering to the teeth after each bleach application. For Ozicure Oxygen Activator the flow meter was constantly monitored to ensure it regulated the ozone gas flow at 5l/min. The absence of leaks around the guards confirmed an optimum seal.

The statistical analysis utilized four of the nine colour readings for investigation of independent variables (24 hour colour). The data set of 396 colour readings was used to gain insights for the descriptive analysis of the study.

4.2. Statistical evaluation of tooth bleaching

Statistical data analysis was done at baseline and each 24 hour tooth colour assessment reading following bleaching. These are said to be the most accurate and stable tooth colour readings to use after tooth bleaching treatment (see section 2.1.7.1). This data set of 396 readings was used to determine the influence of treatment, number of treatments, tooth type and the combination tooth type and treatment on tooth colour change.

Table 4.1 shows the F and P values for the three test groups (Ozicure Oxygen Activator, Opalescence Quick and Control). Tooth colour when assessed via the visual tooth colour assessment method showed significance for independent variables treatment ($F= 9.72$; $P< 0.0001$); number of treatments ($F= 3.41$; $P= 0.0176$); tooth type ($F=3.21$; $P= 0.0416$) and the combination tooth type and treatment ($F= 5.92$; $P= 0.0001$).

Digital colour assessment indicated treatment ($F= 4.34$; $P= 0.0137$) and tooth type ($F= 7.88$; $P= 0.0004$) to be of significance in tooth colour change.

Table 4.1. Comparison of F- and P- values for independent and dependant variables of the study using baseline and 24 hour colour readings following each of the three bleach treatments.

Independent variables	Dependant variable – tooth colour				Number of observations
	Visual tooth colour assessment method		Digital tooth colour assessment method		
	F value	P value	F value	P value	
Treatments: n=3 (Control, Opalescence, Ozicure)	9.72	<0.0001	4.34	0.0137	396
Number of tooth bleaches: n=3	3.41	0.0176	1.35	0.2589	396
Tooth type: n=3 (canine, incisor, premolar)	3.21	0.0416	7.88	0.0004	396
Tooth type and treatment	2.75	0.0280	0.81	0.5209	396

4.3. Descriptive evaluation of tooth bleaching

Graphs have been used to show tooth bleaching trends resulting from the study. The following graphs Figure 4.1 – 4.6 are all laid out in the same way. The x-axis gives the sequence of tooth colour assessments in the study. The y-axis gives the mean tooth colour reading as obtained from the sequential numerical ranking of colour as outlined in sections 4.1.3.1 and 4.1.3.2. As a reminder, visual colour assessment was ranked 1-29 and digital from 1-81, the latter having a greater number of shades which could be discriminated by the instrument. The lightest colour is the lowest number, the darker the tooth the higher the number. Mean values and standard deviations will be given in the text where necessary to clarify detail.

4.3.1. Ozicure Oxygen Activator

4.3.1.1. Digital colour assessment

Mean values for digital tooth colours for Ozicure Oxygen Activator treated teeth broken into tooth type are represented in Figure 4.1. The incisor teeth lightened slightly from baseline to the 24 hour three bleach reading. Both canines and premolars were darker after the three bleaches compared to the first baseline reading. For all three tooth types, colour relapse occurred between the first bleach 24 hour reading and the baseline second bleach tooth colour (canine= 4.5 units; incisor= 4 units; premolar= 2 units). Mean values for first and final colour readings are as follows: canine (from 19.33 ± 12.19 to 22.17 ± 10.48); incisors (from 24.00 ± 10.72 to 23.25 ± 9.10); premolars (from 25.20 ± 13.81 to 26.00 ± 10.72).

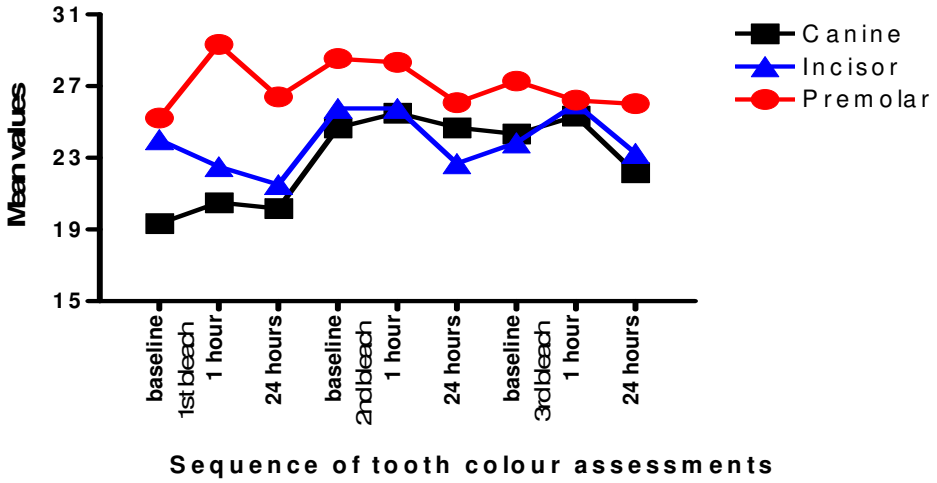


Figure 4.1. Mean values of digital tooth colour for Ozicure Oxygen Activator over the three bleaches.

4.3.1.2. Visual colour assessment

The mean values for visual tooth colours bleached by Ozicure Oxygen Activator broken into tooth type are represented in Figure 4.2. All teeth lightened following bleaching, but little or no further lightening was achieved after the first bleach treatment. Mean values for first and final colour readings are as follows: canine (from 9.17 ± 2.32 to 7.67 ± 3.27); incisors (from 10.67 ± 3.42 to 7.83 ± 2.82); premolars (from 11.47 ± 3.34 to 9.13 ± 3.50).

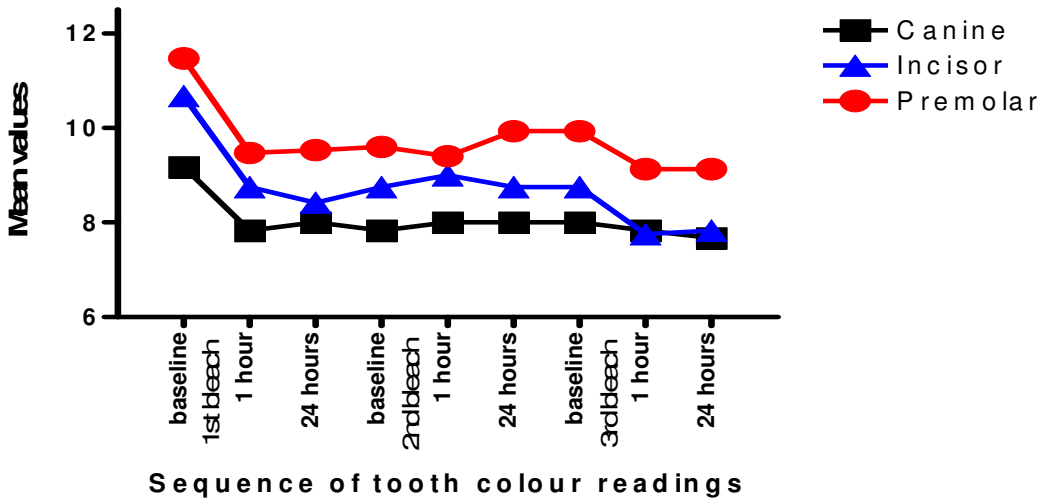


Figure 4.2. Mean values of visual tooth colour for Ozicure Oxygen Activator over the three bleaches.

4.3.2. Opalescence Quick

4.3.2.1. Digital colour assessment

The mean values for digital tooth colours for Opalescence Quick treated teeth are represented in Figure 4.3. While the general trend in the graph is downwards from initial baseline to the final 24 hour reading, indicating a lightening of teeth, the canines and premolars show marked colour swings between the 24 hour first bleach reading and the 24 hour third bleach reading. Mean values for first and final colour readings are as follows: canine (from 26.33 ± 14.22 to 23.17 ± 5.85); incisors (from 23.75 ± 14.12 to 17.83 ± 6.21); premolars (from 32.33 ± 20.01 to 23.07 ± 4.56).

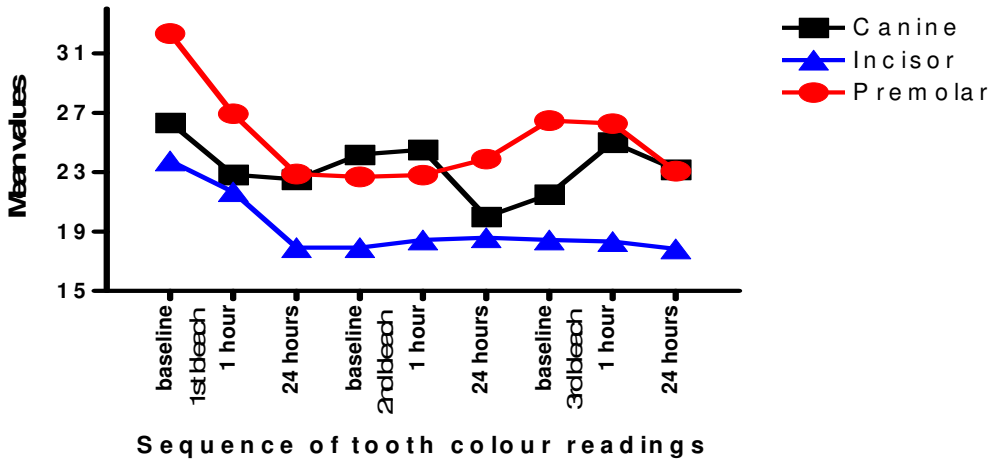


Figure 4.3. Mean values of digital tooth colour for Opalescence Quick over the three bleaches.

4.3.2.2. Visual colour assessment

The mean values for visual tooth colours broken by tooth type for the Opalescence Quick treated teeth are represented in Figure 4.4. As with digital colour reading for this bleach, the colour trend is lighter from initial baseline to final colour. Mean values for first and final colour readings are as follows: canine (from 10.33 ± 4.27 to 7.50 ± 5.01); incisors (from 10.08 ± 3.03 to 7.83 ± 4.34); premolars (from 9.60 ± 2.92 to 7.00 ± 2.07).

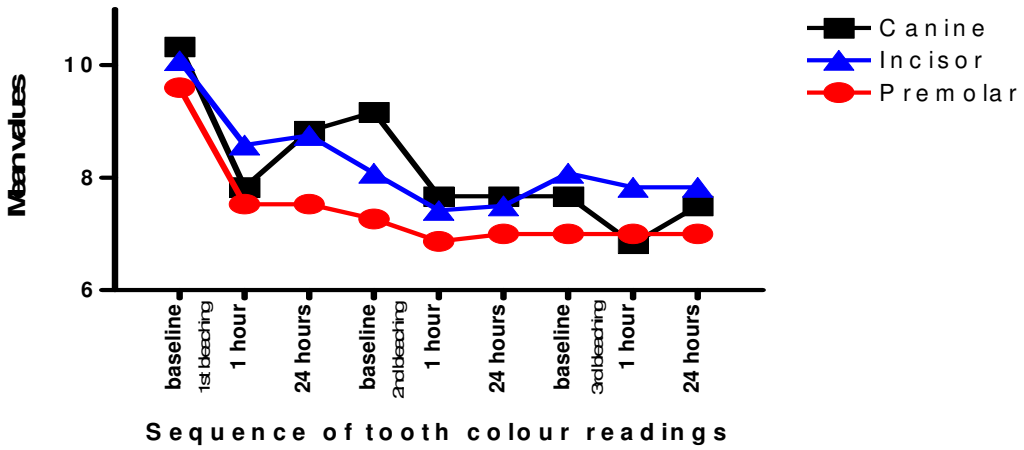


Figure 4.4. Mean values of visual tooth colour for the Opalescence Quick over the three bleaches.

4.3.3. Control

4.3.3.1. Digital colour assessment

Figure 4.5 represents the mean values for digital tooth colour for the control broken into tooth type. The variable colour results are strange and cannot be explained in the light of the control treatment. Section 5.6 discusses problems encountered with the digital color assessment method and a possible answer could lie there. Canine tooth colour varied around the initial baseline mean, while for premolars there was a slight lightening of colour by two and a half units and premolar one unit. In contrast, the incisor teeth lightened in the order of seven shades over the course of the study. Mean values for first and final colour readings are as follows: canine (from 28.67 ± 7.55 to 26.17 ± 5.85); incisors (from 26.25 ± 10.77 to 19.08 ± 4.68); premolars (from 31.07 ± 11.22 to 29.47 ± 13.80).

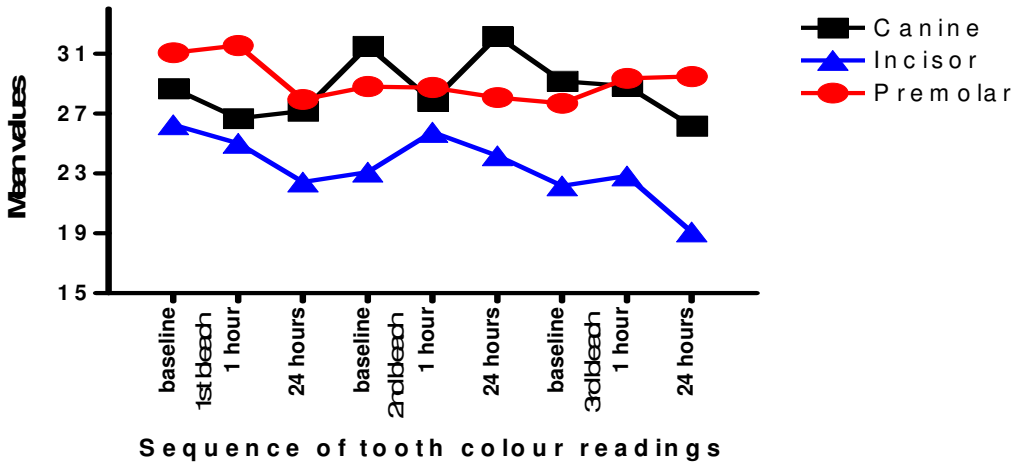


Figure 4.5. Mean values of digital tooth colour for the Control group over the three bleaches.

4.3.3.2. Visual colour assessment

The mean values of the visual tooth colour for the control by tooth type is shown in Figure 4.6. The mean values for canine and premolar tooth colour stayed the same throughout the study. The incisor lightened by half a shade between baseline and first bleach, one hour colour reading. Mean values for first and final colour readings are as follows: canine (from 9.17 ± 1.17 to 9.17 ± 1.17); incisors (from 10.17 ± 3.35 to 10.17 ± 3.35); premolars (from 11.73 ± 4.51 to 11.73 ± 4.51).

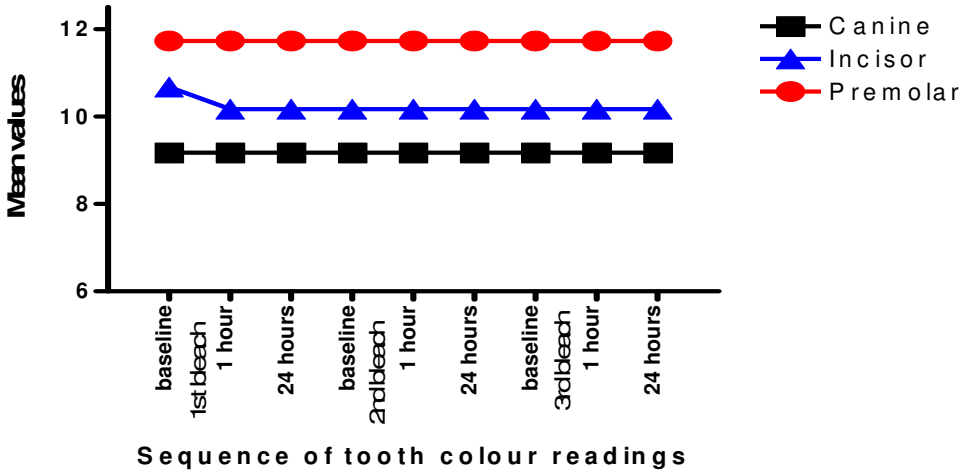


Figure 4.6. Mean values of visual tooth colour for the Control over the three bleaches.

Two observations can be made from this section of the results. As a generalisation, bleaching caused the teeth to lighten. Secondly, the six graphs show very clearly the instability of tooth colour over the course of the investigation. This will be further explored in the next section.

4.4. Tooth colour relapse

Colour relapse was deemed to have occurred if a tooth was one shade darker that the previous reading. Table 4.2 summarises relapse in tooth colour at two intervals in the study. These are (a) between the 24 hour first bleach and baseline colour before the start of the second bleaching treatment and (b) between the 24 hours tooth colour after the first bleaching and final tooth colour (24 hours after third bleaching). The Table 4.2 shows that visual colour relapse is less than the measured digital colour relapse. Finally, tooth colour relapse was particularly noticeable digitally, when 24 hours tooth colour after the first and final tooth colour (24 hours after third bleach) was compared.

Table 4.2. Summary of tooth colour relapse occurring at two stages in the study.

Treatment group	Visual colour assessment (Tooth colour relapse %)	Digital colour assessment (Tooth colour relapse %)
(a) Colour relapse: comparing 24 hour tooth colour after first bleaching with baseline tooth colour before second bleaching treatment.		
Ozicure Oxygen Activator	9%	33%
Opalescence Quick	6%	21%
(b) Colour relapse: comparing 24 hour tooth colour after first bleaching with final tooth colour.		
Ozicure Oxygen Activator	9%	54%
Opalescence Quick	9%	18%

4.5. Tooth specimens showing no change in colour

All teeth assessed digitally showed a colour change from the initial baseline colour to the final colour assessment. Tooth specimens showing no colour change were limited to the visual colour assessment group. The results of this sub-group are summarized in Table 4.3. The teeth resistant to colour change were mainly canines and belonged to the Ozicure Oxygen Activator treatment group. The teeth that did not show any change in tooth colour represent 9.8% of all the teeth in this bleaching study. Control teeth are shown in Table 4.3 for completeness.

Table 4.3. Teeth showing no visual colour change after three bleaching treatments.

Treatment Groups	Canine n=18	Premolar n=45	Incisor n=36	% of teeth
Opalescence Quick	2	3	0	15%
Ozicure Oxygen Activator	2	5	1	24%
Percentage of teeth	22% of canines	17% of premolars	2.7% of incisors	
Control	11	11	10	97%

4.6. Data interactions to elucidate hypotheses

In the next section, statistical and descriptive data will be used to elucidate hypotheses listed in section 2.4. The statistical programme using repeated measurements on the same specimen cannot arrange variables from best to worst. This can only be deduced by comparing mean values and standard deviations based on the ranked numerical colour shade values.

4.6.1. Tooth colour change with Ozicure Oxygen activator is superior to that obtained with Opalescence Quick

4.6.1.1. Visual tooth colour assessment

The GLM test for Analysis of Variance for a Fractional Design showed there was statistically no significant difference between tooth colour change obtained with either bleaching agent (F= 2.45; P= 0.1193). However a significant

difference was apparent when Ozicure was compared with the control (F= 9.78; P= 0.0021) and Opalescence compared with the control (F= 24.94; P< 0.0001). The visual colour assessment cannot distinguish between tooth colour change with Opalescence and Ozicure.

Mean values and standard deviations show that colour lightened by approximately two and a half shades overall for each product: Opalescence Quick tooth colour for the first observation was 9.91± 3.14 and for the last observation was 7.39± 3.53. Likewise, Ozicure Oxygen Activator went from 10.76± 3.23 to 8.39± 3.20.

4.6.1.2. Digital tooth colour assessment

Statistically a similar pattern emerged in the case of digital tooth colour assessment. There was no significant difference between tooth colour change obtained with either bleaching agent (F= 2.68; P= 0.1036). The same occurred when Ozicure was compared with the control (F= 2.27; P= 0.1340). A significant difference was apparent when Opalescence was compared with the control (F= 11.35; P< 0.0009).

However while the mean values for Opalescence Quick declined from 28.12± 17.07 (first observation) to 21.18± 5.85 (final observation), Ozicure Oxygen Activator mean values rose from 23.70± 12.28 (first observation) to 24.30± 9.92 (final observation).

In light of these contradictory results this study has been unable to show which is the superior bleaching agent between the two products studied.

4.6.2. Canine, incisor and premolar teeth respond differently to bleaching.

Statistically there was a significant difference between tooth colour and tooth type for both methods of colour assessment (Table 3.1). But this does not give the bleaching response of each individual tooth type. To explore this aspect of the study the GLM test was run selectively by tooth type for both colour assessment methods. In each case the baseline colour value (first reading) was run sequentially and individually against each following reading until a significant difference in colour was obtained.

Incisor teeth lightened significantly visually from the sixth colour assessment onwards ($F= 4.31$; $P= 0.0147$). The sixth colour assessment being the second bleach, 24 hour assessment. Digitally the incisor tooth colour changed significantly from the fifth colour assessment onwards ($F= 3.41$; $P= 0.0460$). The fifth colour assessment being at one hour after the second bleach.

Canine tooth colour did not change significantly throughout the study for visual colour assessment. Digitally the colour changed from the sixth colour assessment onwards ($F= 3.20$; $P= 0.0455$). The sixth colour assessment being the second bleach, 24 hour assessment.

Premolar teeth showed a significant tooth colour change (visually) from the

second tooth colour assessment (F= 5.37; P= 0.0064). The second colour assessment being one hour after the first bleach. Digitally significant tooth colour change was seen at the ninth tooth colour assessment (F= 3.20; P= 0.0420). The ninth colour assessment is the final 24 hour, third bleach assessment.

These results show that the different tooth types studied respond differently to bleaching.

4.7. Comparison of tooth colour assessment methods

The comparison between digital and visual tooth colour assessments is most conveniently summarised in terms of time, ease of use, accuracy and cost.

Training of the observer to ensure accurate visual colour assessment occurred via the intra-examiner test. It took approximately 10 minutes to do a visual tooth colour assessment of one replicate of 11 teeth. In contrast the same number of teeth took 25 minutes for digital tooth colour assessment.

The digital method of assessment was so time consuming because it is recommended by the manufacturer that a tooth colour must be confirmed three times for accuracy. The before mentioned may be as a result of the quality of the instrument.

However, in practice, individual tooth colour results varied over the full spectrum of all available tooth colours. This meant that individual tooth colour

results had to be performed more than four times or more for confirmation. In addition it was necessary to re-calibrate the instrument by switching off and on again to permit self-recalibration before continuing with tooth colour assessment. This necessary re-calibration has raised doubts about the accuracy of the tooth colour readings recorded by the Vita Easyshade.

At the time of the study the price tag for the Vita Easyshade was R 37 141.20 versus R 1 026.68 for the VITAPAN 3D MASTER TOOTH GUIDE (VITA, Germany). This discrepancy in price may deter the dentist from buying this product.

4.8. Tooth enamel surfaces changes

4.8.1. Enamel mineral loss

Examination of the specimen at high magnifications showed no enamel erosion between the varnish covered and exposed tooth surfaces following bleaching treatment. Similarly no change in enamel prism arrangement or opacities could be observed between exposed and unexposed tooth enamel (Figure 4.7).

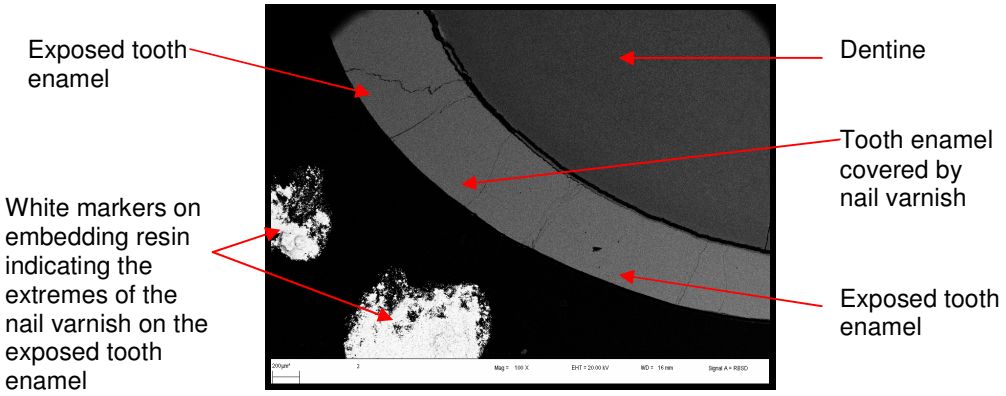


Figure 4.7. SEM micrograph showing a varnished tooth enamel area bordered by adjacent exposed tooth enamel.

The Ca:P ratios were determined on both exposed and control areas (enamel covered with nail varnish). There was no apparent difference within experimental error between the Ca:P ratios between the nail varnish covered regions and the neighbouring exposed enamel in the Opalescence Quick group (Table 4.4). Some indication of the errors involved can be estimated from the control data at the far right hand of Table 4.4. The Ca:P ratio for the Ozicure Oxygen Activator (tooth with least colour change) was 2.24:1 (control). While the Ca:P ratio for the exposed enamel was 2.44:1. This indicates a change that may be an acceptable natural variance or due to the experiment.

Table 4.4. Ca:P ratios of unexposed (control) tooth enamel and exposed tooth enamel for the three treatments showing least and most colour change.

Experimental groups	Unexposed (control) enamel			Exposed enamel			Error
	Ca wt%	P wt%	Ca:P ratio	Ca wt%	P wt%	Ca:P ratio	
Opalescence Quick							
Least colour change	29.4	17.1	1.72:1	28.8	16.8	1.71:1	0.01
Most colour change	37.8	18.3	2.06:1	37.3	18.5	2.02:1	0.04
Ozicure Oxygen Activator							
Least colour change	40.5	18.1	2.24:1	47.6	19.5	2.44:1	0.2
Most colour change	33.2	17.4	1.91:1	33.1	17.5	1.89:1	0.02
Control							
Least colour change	34.1	17.5	1.95:1	35.8	18.8	1.90:1	0.05
Most colour change	38.6	18.4	2.10:1	37.4	18.4	2.03:1	0.02

In summary there was no visible morphological change in tooth enamel, nor an alteration in enamel mineral distribution to indicate an erosive effect of bleaching on the enamel tooth surface.

4.9. Surface roughness

The possible effects caused by the two bleaching agents (Opalescence Quick and the Ozicure Oxygen Activator) on the enamel surface of the teeth were investigated. The tooth surface effects were examined using the AFM.

For each of the specimens two figures will be shown representing a typical result of the treatment. The first will be a micrograph taken at a standard magnification over a square of side length of 10μm in both the X and Y directions. The coloured bar to the left of each AFM micrograph indicates depth; the darker areas indicating a valley and the lighter area a peak (see for example Figure 4.8). The vertical numbered bar scale should be ignored as it does not reflect the true calibration of the instrument.

The second figure is a histogram representing the surface roughness of the specimen. Important features on each histogram are the height, given at the top of the peak, the horizontal distance between the peak and the left and the right tails respectively, which indicate the regularity of the surface.

4.9.1. Control group

The normal, unbleached enamel tooth surface shows many irregularities as can be seen in Figure 4.8. Long, dark, scratch-like marks are seen in the AFM micrograph.

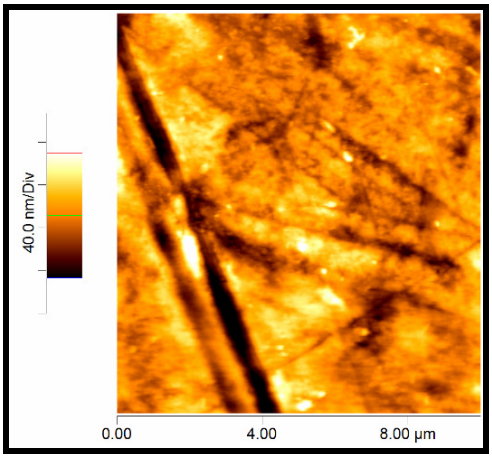


Figure 4.8. AFM micrograph of the non-bleached tooth surface. The study was carried out over a square of side length of 10µm in the X and Y directions. The average surface roughness was 14.7nm. The dark lines on the micrograph represent typical scratch-like markings found on human extracted teeth. These markings are the result of masticatory wear and tear.

Figure 4.9 is derived from the surface roughness at each pixel of the scanned surface. The horizontal scale indicates height of the feature, while the vertical scale gives the frequency for a given height in the entire 10 x 10 micrometre image. Numerical numbers at the top are meaningless. The histogram represents a skew Gaussian distribution for the scanned non-bleached tooth (the vertical red and blue lines have no meaning). The peak to tail length on the left side of the histogram is longer than the peak to tail of the right side of the histogram. This means there are many small peaks and valleys dominated by fewer, but greater peaks and valleys. This may be probably due to the scratches seen in the micrograph (Figure 4.8). The skew Gaussian for the enamel tooth surface (Figure 4.9) means that specimen roughness/topography is dominated by roughness smaller than that corresponding to the peak of the histogram.

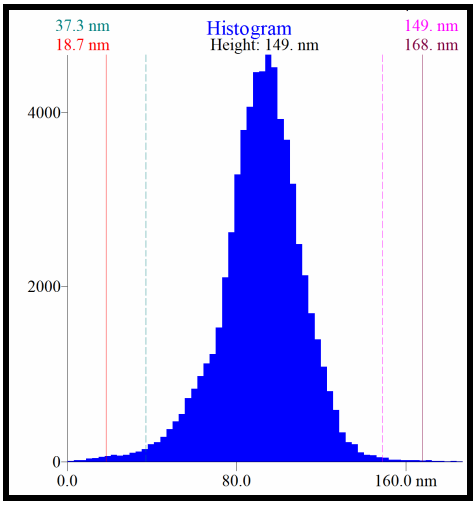


Figure 4.9. Histogram of the surface roughness of the non-bleached tooth surface area showing a left skewed Gaussian distribution.

4.9.2. Ozicure Oxygen Activator

The surface roughness was found to be more regular following the Ozicure Oxygen Activator bleaching (Figure 4.10).This distribution represents a more even tooth enamel surface compared to the unbleached surface, Figure 1.8, with a narrower depth margin between the valleys and the peaks as indicated by the colour bar scale to the left of the micrograph.

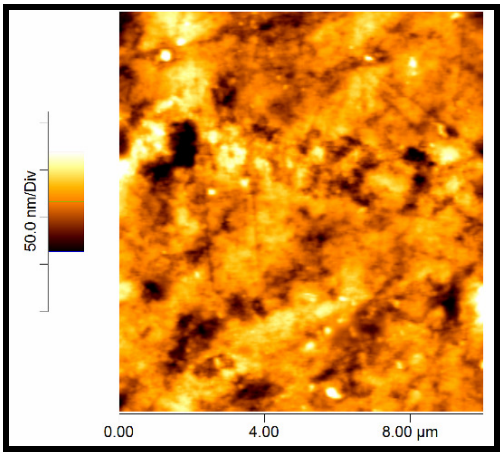


Figure 4.10. AFM micrograph of a tooth surface bleached with the Ozicure Oxygen Activator. The study was carried out over a square of side length of 10µm in the X and Y directions. The average surface roughness was 13.3nm.

In Figure 4.11, the distance between the peak and the tail on the right and left side of the peak is roughly the same, but very slightly skew towards the left. The Ozicure Oxygen Activator bleaching produced a more bell-shape or symmetrical Gaussian distribution for the surface roughness meaning that the roughness is symmetrical about that corresponding to the peak value.

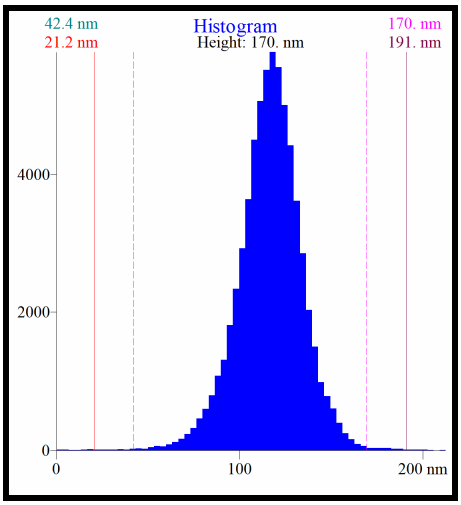


Figure 4.11. Histogram of the surface roughness showing a Bell-shape or a symmetrical Gaussian distribution of the bleached tooth surface area (Ozicure Oxygen Activator).

4.9.3. Opalescence Quick

The Opalescence Quick bleaching procedure produced a rather rougher tooth surface (Figure 4.12) compared to the control. The average surface roughness was 32.6nm. Opalescence Quick produced almost a 2.5 times rougher enamel tooth surface than the Ozicure Oxygen Activator and the control.

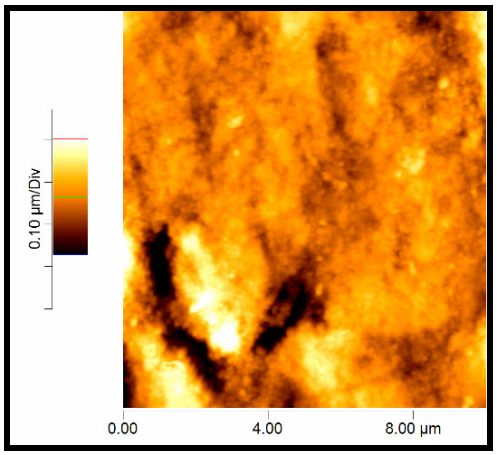


Figure 4.12. AFM photograph of the bleached tooth surface after bleaching with Opalescence Quick. The study was carried out over a square of side length of 10µm in the X and Y directions. The average surface roughness was 32.6nm.

While the distribution is roughly a symmetrical Gaussian distribution (Figure 4.13), the distribution is less Gaussian than for the Ozicure Oxygen Activator and thus ultimately it has more surface roughness than Ozicure Oxygen Activator.

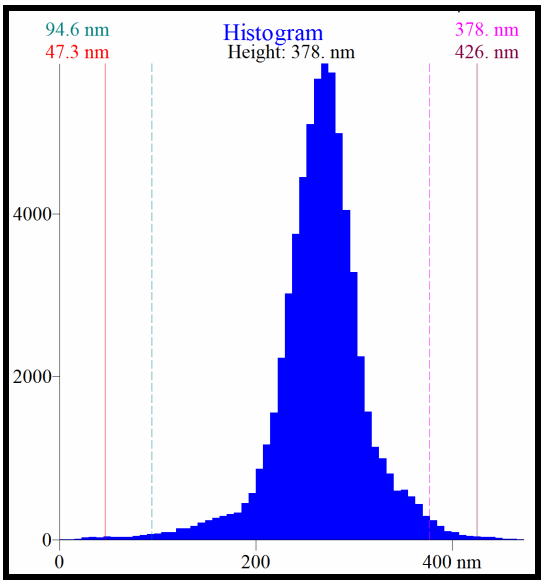


Figure 4.13. Histogram of the surface roughness showing a bell-shape or a symmetrical Gaussian distribution of the bleached tooth surface area (Opalescence Quick).

In summary, the surface roughness results show that Opalescence Quick caused the highest surface roughness on the exposed enamel surfaces. Both bleaching systems produced a more regular surface than the initial samples as seen by the Gaussian symmetry compared to that of the control.

Chapter 5 Discussion

This study was undertaken to examine the bleaching potential of a new, in-office tooth bleaching product Ozicure Oxygen Activator (O_3, RSA). In order to do so it was compared to Opalessence Quick (35% carbamide peroxide).

5.1. Tooth lightening

Both bleaching methods resulted in a statistically significant lighter final tooth colour, visual ($P<0.0001$) and digitally ($P=0.0137$). The statistical programme using repeated measurements on the same specimen cannot indicate which bleaching treatment method was the best. However this could be deduced by comparing mean values and standard deviations based on the ranked numerical colour shade values. Opalescence Quick (Ultradent, USA) performed slightly better than the Ozicure Oxygen Activator (O_3, S.A) bleaching system based on visual colour assessment. Digitally, the result is contradictory. Descriptive statistics indicate that greater colour relapse and a greater number of teeth resistant to colour change were found in the Ozicure group. This does not mean that Ozicure is an inferior bleach, statistically both bleaches lightened the teeth significantly in the study. The slight difference in bleaching performance of the two products could lie in the differing concentrations of bleach. In the case of Ozicure Oxygen Activator the Trèswhite strips contained 9% hydrogen peroxide. The gel of Opalescence Quick contains 35% carbamide peroxide, but as pointed out in section 1.3.1. it is unclear how much active agent is produced by this compound.

5.2. Application frequency of the bleaching agent

The General Linear Models test for Analysis of Variance for a Fractional Design indicated that visual colour assessment showed significance with the number of bleach treatments, digitally this was not the case. The descriptive results are ambiguous and appear to indicate that after the first hour of tooth bleaching no, or little, benefit was achieved with repetitive bleaching. However interactive statistics show that significant tooth colour change occurred in a range measured at one hour after the first bleach to the final colour reading, depending on what tooth type was examined. A statistically significant change in tooth colour translated to one shade tab (unit) for visual and one shade tab (unit) for digital assessment. Clinically a one shade lightening in colour is considered a “success” according to the author. The results of the present study are unable to confirm the findings of Rosentiel *et al*., (1991) and Kielbassa et al., (2009) that multiple bleaching treatments are of limited value.

5.3. Tooth type

In this study, the three different tooth types responded differently to the bleaching treatments. This was confirmed statistically using both visual and digital colour assessment. Linked variables “tooth type treatment” (Table 4.1) was only significant with visual colour assessment indicating that treatment type plays a role in tooth type response. The interactive statistics show that visually premolars change colour significantly one hour after the first bleach. This study confirmed the results of Leonard et al., (1998) that tooth type played a significant role in tooth colour change and the different tooth types responded differently to the bleaching treatments. However in the present

investigation there were unequal numbers of the different tooth types (canines = 18; premolars= 45; incisors= 36) and this may have played a role in the statistical outcome of this part of the investigation.

5.4. Tooth colour stability and relapse

Tooth colour relapse is a normal complication found during a bleaching treatment and a few studies (Gerlach *et al*., 2002; Mohkhlis *et al*., 2000) mention this complication. Gerlach *et al*., 2002 reported tooth colour relapse after tooth bleaching, but failed to specify the amount of tooth colour relapse recorded.

Evaluating colour relapse is complicated by three issues: determining a time interval between which to measure relapse; the threshold number of shades beyond which relapse is considered a problem and the acceptable number of teeth relapsing within a specific treatment regimen. Such an evaluation is further complicated in this study by the two colour assessment methods where digitally 81 shades were measured as opposed to the 29 shade tabs of the visual method. Clearly a one shade difference measured digitally cannot be equated with a visual one shade difference.

Kielbassa *et al*., (2009) maintains that the colour reading taken 24 hours after bleaching is the most stable. The visual colour assessment method in the present study confirmed Kielbassa *et al*., (2009) findings. However this present study based colour relapse on a colour darkening by one shade. The digital results of this study revealed a high percentage of tooth colour relapse,

irrespective of which time intervals were compared. The percentage of tooth colour relapse digitally is definitely greater than the two shade relapse reported by Mokhlis *et al.*, 2000). This may be an indication of the method rather than the bleaching procedure.

This study indicates that a fairer assessment of colour relapse may be the colour change occurring in the interval 24 hours after the first bleach compared with the baseline tooth colour (second bleach).

In summary a greater colour relapse and a greater number of teeth resistant to colour change were found in the Ozicure group. The 6-9% tooth colour relapse found with visual colour assessment was clinically acceptable according to the researcher.

5.5. Teeth showing no colour change

One can only speculate as to why certain teeth in the present study did not show any tooth colour change. It could be that these specific teeth were not bleached long enough. Alternatively the bleach did not reach the stain or thirdly the stain type of the tooth was resistant to the bleach. There were no reports in the literature covered which mentioned teeth resistant to bleaching.

5.6. Tooth colour assessment methods and ease of use

Horn *et al.*, (1998), reported that intra evaluator agreement ranged from twenty to sixty percent in their study. Alternatively, Watts and Addy (2001) maintain that training improves visual colour assessment skills of a dentist and is

essential for reliable results. The visual colour assessment method using the VITAPAN 3D MASTER TOOTH GUIDE (VITA, Germany) was fast and easy to use. One of the limitations of the present study is that the visual colour assessments were performed only by one researcher and that bias could have crept into the study. However Kielbassa *et al*., (2009), in a study which similarly used a single colour assessor, extensively discusses the options of single versus more than one evaluator. They concluded that inter-and intra-evaluator disagreements can be equally limiting to the outcome of a study.

The digital colour assessment method (VITA Easyshade, Germany) was time consuming and problematic. The method does not produce a consistent tooth colour after three assessments carried out as indicated by the manufacturer. Instead, tooth colour was given over the whole tooth colour spectrum available. Throughout the study, the digital colour assessment method showed many inconsistencies within itself. As an example, the outcome of digital assessment of control teeth was particularly perplexing (Fig 4.5). The results for digital tooth colour assessment in this research report were in contradiction with previous studies that claimed digital tooth colour assessment methods were superior to visual tooth colour assessment (Horn *et al*., 1998; Kielbassa *et al*., 2009). The deduction can be made that the quality of the above spectrophotometer is questionable.

In the present study visual tooth colour assessments were faster and easier to do than using the digital colour assessment method. However accuracy of measurement with digital assessment was suspect.

The costs associated with the digital method were around 40 times greater than that for the standard visual method. The digital method for tooth colour assessment is a promising new product for the future. The results from the specific instrument used in this *in vitro* study may be questionable due to the quality there of.

At present, there is no clear indicator for an acceptable clinical colour assessment error. It seems to be subjective, influenced by the clinician's and patient's perception of the right tooth colour for the specific individual. The question remains what margin of error exists that is still clinically acceptable when using a digital method of tooth colour assessment.

5.7. Tooth surface changes

The most fascinating finding in this part of the study was that both bleaching systems created a more regular surface compared to the control. What this means is unknown and worthy of further exploration. Opalescence Quick bleached tooth enamel surface roughness increased when compared with that of normal tooth enamel and Ozicure Oxygen Activator surfaces. Increased roughness implies surface loss, but paradoxically this was not accompanied by a change in Ca:P ratios (Opalescence Quick group). It could be that the method used to measure calcium and phosphate was not sensitive enough for this study, that is, the electron beam detected too far into the tooth. The data of the Ozicure Oxygen Activator method does highlight the difficulties in this analysis since the least colour change ratio altered by 10 percent compared to one percent for the teeth with the most colour change. Other authors

(Sulieman *et al*., 2004; Lopes *et al*., 2002) have also reported no surface changes during bleaching.

The Ca:P ratio (Ozicure Oxygen Activator) was 2.2:1 for the control, with 40.5 wt% calcium and 18.1 wt% phosphate. The Ca:P ratio for the exposed enamel surface was 2.4:1, with 47.6 wt% calcium and 19.5 wt% phosphate. This difference indicates a change, which could be an acceptable natural variance or due to the experiment.

Surface roughness measurements on enamel are seldom done and the reason maybe that the curvature of the teeth makes measurements problematic. Only one previous study investigated surface roughness of enamel after a bleaching treatment (Hegedüs *et al*., 1999). These investigators found that the enamel surface treated with bleach became irregular and rougher than untreated enamel. This *in vitro* study found normal enamel tooth surfaces irregular and tooth bleaching with Opalescence Quick produced a 2.5 times greater surface roughness than the Ozicure Oxygen Activator.

The clinical relevance of this new finding is unclear at this stage. One can only speculate that increased roughness could facilitate bleach penetration into the enamel surface. Since Opalescence Quick was marginally the better bleach, it is convenient, but hardly scientific to link the two. Equally a rougher surface could alter light paths resulting in apparent tooth lightening.

The results of the AFM study revealed a decrease in the difference between the valleys and peaks of the enamel tooth surface after treatment with the

bleaching agents. This may be interpreted as a decrease in surface roughness. In the current study the teeth were treated for three hours in total and is in close relation to the clinical application. In the study by Hegedüs *et al*., (1999) the teeth were treated for 28 hours, this is a very long time for bleaching with a high concentration bleaching agents. It is thus not surprising that their results revealed surface changes. The micro-hardness study by Majeed *et al*., (2008) also implies tooth material loss following 112 hours of tooth bleaching.

Larger, longer time and more studies have to be undertaken to explore these promising findings.

Chapter 6 Conclusions

In this section, the conclusions of the study will be drawn according to the hypothesis presented in section 2.4.

1. Tooth colour change with Ozicure Oxygen Activator is superior and more stable to that obtained by Opalescence Quick.

The statistical tests cannot provide clear results as to which bleaching system is superior, but the results show that both systems bleach teeth. From the mean values, Opalescence Quick produced a marginally lighter tooth, while greater colour relapse and a greater number of teeth resistant to colour change were among the Ozicure bleached teeth.

2. The three anterior tooth types (canine, incisor and premolar) respond differently to bleaching.

The present *in vitro* study confirmed previous results (Leonard *et al*., 1998) that teeth respond differently by tooth type, when bleached under the same conditions.

3. Teeth will become progressively lighter with ongoing bleaching.

The descriptive and statistical results do not entirely support the contention made by Rosentiel *et al*., (1991) that after the first bleaching treatment there is little or no benefit with repeated bleaching treatments.

4. Tooth colour assessment methods will reflect a similar trend of tooth bleaching outcomes between the two bleaching agents.

Of the three independent variables, treatment and tooth type were both indicated as being statistically significant in tooth colour, no matter the tooth colour assessment method. Visual tooth colour assessment indicated that the number of tooth bleaches was an additional statistically significant factor in tooth colour assessment, which was not the case in digital colour assessment. As such, the tooth colour assessment methods were not in total agreement with tooth bleaching trends.

5. The bleaching process does not cause any change to the enamel tooth surface in terms of surface roughness or tooth surface loss (as measured by Ca:P ratios).

There was no apparent tooth surface loss in terms of Ca:P ratios between bleached and unbleached enamel surfaces. No morphological changes were apparent when specimens were examined with the SEM. In contrast, AFM analysis indicated that Opalescence Quick produced a 2.5 times rougher enamel surface than the control and Ozicure Oxygen Activator treated specimens. Bleaching resulted in a more regular enamel surface than the control surface as revealed by Gaussian symmetry. Future investigations need to explore the above findings and to determine their clinical relevance. Examination of surface roughness over larger regions would be extremely beneficial.

Chapter 7 Short-comings of the study and future suggestions

With hindsight the following variables contributed to limitations in the present investigation. It would have been helpful if these had been known and addressed beforehand to ensure a more refined study design.

- It may have been a better idea to assign certain tooth colours into groups or classes to narrow down the difference in the number of tooth colours available between the VITAPAN 3D MASTER TOOTH GUIDE and the VITA Easyshade. This would have narrowed down the available tooth colour options between the two colour assessment methods and may have produced better statistical correlation.

7.1. Future investigations and unanswered questions

As is the nature with such investigations more unanswered questions have arisen than could be answered. The following future investigations are suggested to tackle unanswered questions:

- The reason why some teeth respond to bleaching treatments and lighten considerably and other teeth show no change creates an important question. More detailed and area specific surface roughness and Ca:P ratio studies, looking at mineral loss may help with this question. This could involve area markers made of nano-indentation methods to enable precise site specific studies. Such investigations should preferably include measurements after the first and second

bleaching treatments. The current study only looked at surface roughness and Ca:P ratios after three bleaching treatments. A more localized and detailed, area specific surface sensitive compositional analysis technique would seem to be more appropriate than the EDX method utilized here, since the EDX signal in this study emanated from tooth up to about 3.0µm below the surface. X-ray photoelectron spectroscopy, XPS, (also known as ESCA) analysis of the surface as the surface is systematically eroded away (depth profiling) would give an indication of the compositional changes with depth. Such analysis has an escape depth of only 1-10nm with a detection limit of 0.1 at%.

- The variable surface roughness caused by the bleaching agents in this study results raises intriguing questions. Does the surface roughness caused by the bleaching agent have any clinical relevance and why does Opalescence Quick produce a higher surface roughness compared to Ozicure Oxygen Activator and the control? Are the surface roughness results for Opalescence Quick clinically relevant? Does it cause surface damage or compositional modification, resulting in tooth lightening. The symmetrical Gaussian distribution of bleached surface enamel compared to the control raises further questions about the effect which bleaching has on enamel at the sub-microscope level.

- The present study has pointed out that further studies need to be undertaken using bigger sample sizes when mineral loss and surface roughness after bleaching treatments are investigated. In addition, surface roughness measurements over larger areas would be an advantage to improve statistics.

- Further work needs to establish what total bleach time will cause surface changes on enamel tooth surfaces and even more intriguingly after what bleaching time does the change become irreversible.

- From this study, it is not clear whether the Ozicure Oxygen Activator or the Trèswhite strip or both together were responsible for tooth bleaching. Tooth bleaching with the Ozicure Oxygen Activator, with or without the Treswhite strips has to be tested.

- The response of specific stain types to bleaching must be investigated.

- What is the relationship between bleach penetration, stain removal and tooth enamel morphology

7.2. Study recommendations

- While it is understood that the bleaching guard and the seal it creates during the Ozicure Oxygen Activator bleaching process are essential factors in bleaching efficiency, the bleaching method is laborious and cumbersome. The system would benefit from significant simplification and user friendliness by manufacturing a dual guard with the following requirements: it must be possible to use the guard in all patients' mouths and acquire an optimal seal.

- The dentist may not be so enthusiastic switching to the digital tooth colour assessment method taking into account the huge cost difference between the VITAPAN 3D MASTER TOOTH GUIDE (VITA, Germany) and the VITA, Easyshade (VITA, Germany) and extra time required in its utilization. Many improvements may be needed on the VITA Easyhade (VITA, Germany) to the justify the price tag.

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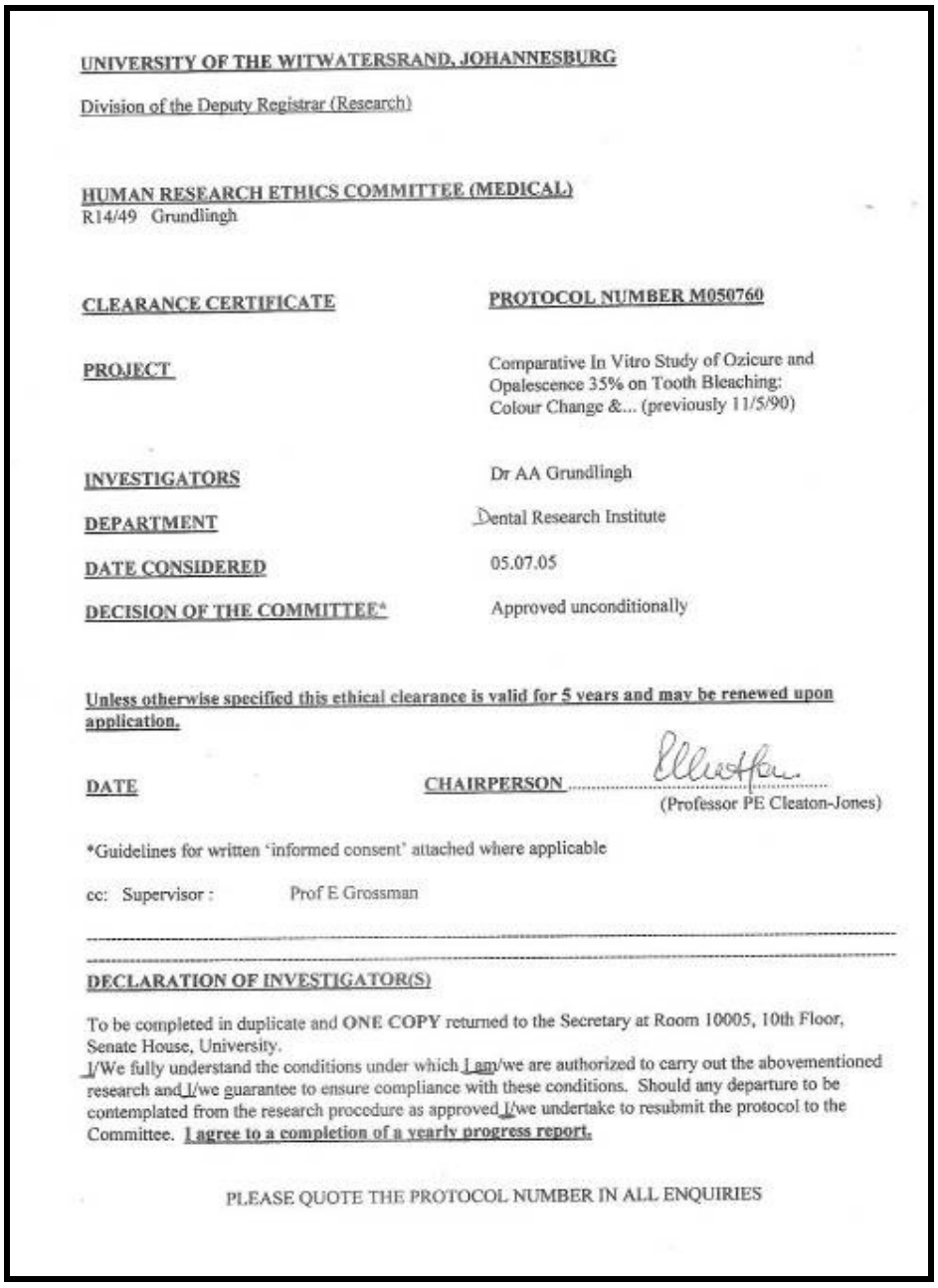
Appendices

Pilot studies

Pilot studies were undertaken to refine and finalise the methodology undertaken in this research report. Each individual pilot study and a copy of the ethics clearance certificate can be found in the following Appendices.

Appendix A: Ethics clearance certificate

An ethics clearance certificate (M050760) was obtained through the HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL) at the University of the Witwatersrand, Johannesburg for the use of human extracted teeth.



UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Grundlingh

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M050760**
PROJECT	Comparative In Vitro Study of Ozicure and Opalescence 35% on Tooth Bleaching: Colour Change &... (previously 11/5/90)
INVESTIGATORS	Dr AA Grundlingh
DEPARTMENT	Dental Research Institute
DATE CONSIDERED	05.07.05
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof E Grossman

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B: Obtaining a control surface for SEM and AFM studies

Rationale

A valid comparison is required to evaluate any enamel surface alterations caused by bleaching. The natural tooth surface varies between individual teeth and therefore nail varnish is often used to cover certain tooth areas prior to bleaching to protect that surface from the treatment. Once the nail varnish is removed, it creates a control surface for comparison with the bleached surface. In this way each tooth acts as its own control.

What is the problem?

Coloured nail varnish is visible and is easy to remove, but may interfere with tooth colour assessment.

Options to solve the problem.

The aim of the pilot study was to see if red or clear nail varnish caused any interference with tooth colour assessment.

Methods used.

Three teeth were used in the study: a canine, a premolar and an incisor tooth. Tooth colour was taken at baseline using the VITAPAN 3D MASTER TOOTH GUIDE (VITA, Germany). Thereafter the palatal aspect of the teeth was covered with clear nail varnish and left to dry for one hour (Figure B.1). The border of the clear nail varnish was lightly marked with a graphite pencil and colour assessment was made one and 24 hours after the nail varnish was applied (Table B.1). The clear nail varnish was removed with acetone and the red nail varnish was applied within the pencilled marks. Colour assessment was repeated as before. The researcher was blinded to all previous tooth colour readings.

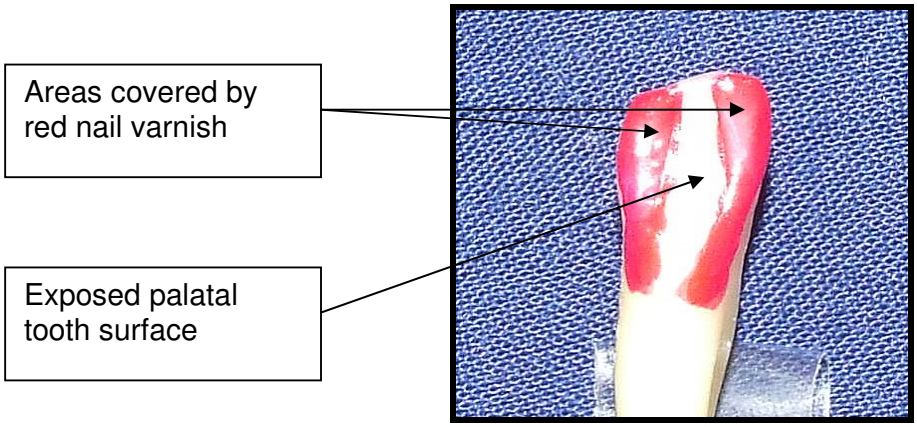


Figure B.1. Red nail varnish covering the palatal aspect of the incisor tooth.

Outcome of options.

Red varnish affected the tooth colour assessment of the incisor teeth, but not the canine or premolar. In Table B.1, the change in colour is highlighted in red. Clear nail varnish on the other hand did not result in a change in tooth colour.

Table B.1. Red and clear nail varnish colour results.

Nail varnish	Tooth type	Tooth number	Tooth colour		
			Baseline	1 hour	24 hours
	Canine	1	2L2.5		
Clear	Canine	1		2L2.5	2L2.5
Red	Canine	1		2L2.5	2L2.5
	Incisor	2	2L2.5		
Clear	Incisor	2		2L2.5	2L2.5
Red	**Incisor**	**2**		**2R2.5**	**2R2.5**
	Premolar	3	2M3		
Clear	Premolar	3		2M3	2M3
Red	Premolar	3		2M3	2M3

Final decision on the way forward.

Clear nail varnish was indicated as the preferred material to use in order to create a control surface.

Appendix C: Pilot base model and bleaching guard fabrication

Rationale

The bleaching study required the extracted teeth to be kept stationary during the experimental procedures and required a base model to be devised. The model had to ensure maximum contact between the bleaching agent and the teeth. Furthermore each tooth had to have its own specific individual positions on the base model to prevent the teeth from being mixed up.

What is the problem?

The model devised had to be robust, reusable, repeatable and as close as possible to the clinical situation. It also had to cater to each bleach treatment and required sockets specific for each tooth so that each was always in the same position.

Options to solve the problem.

Pilot base models were made to keep the teeth and bleaching guard stationary during the bleaching procedure for both Opalescence Quick (Figures C.1 and C.3) and Ozicure Oxygen Activator (Figures C.4 and C.6). This also ensured maximum contact between the bleaching agent and the teeth.

Two base models were made from pink wax [Kemdent Modeling wax no: 4 (S.A.)]. On each model a wax up of three teeth was done. The base models went through a pack and cure process after the teeth were removed to prepare the base models for finishing (Figure C.1 and C.4). The teeth were tried for fit in their individual positions on the base models (Figure C.2 and C.5). Bleaching guards made with Ministar S (SHEU, Germany) fitted tightly over the base models. The Opalescence Quick bleaching guard was two millimetres thick (Figure C.1), one millimetre thicker than the instructions of the manufacturer. The added thickness provided superior retention of the bleaching guard. The Ozicure Oxygen Activator bleaching guard was made according to the manufacturer's instructions, three millimetres thick (Figure C.4).

Opalescence Quick



Figure C.1. Opalescence Quick base model and bleaching guard used for the pilot study.



Figure C.2. The teeth were placed in their individual positions on the base model.



Figure C.3. The teeth were covered with the bleaching guard containing the bleaching agent Opalescence Quick.

Ozicure Oxygen Activator



Figure C.4 Ozicure Oxygen Activator base model and bleaching guard with inlets for the ozone gas.



Figure C.5. The teeth were placed in their individual positions and wetted with activated water.



Figure C.6. The bleaching guard was placed in position and attached to the Ozicure Oxygen Activator.

Another problem that came out from the pilot study.

The teeth could be negatively affected by heat during the fabrication of bleaching guards.

Solving the problem.

A stone model is usually used to overcome this problem. Consequently, a custom wooden frame was made to make an impression of the base models with the teeth. Yellow stone was poured into the impression and the stone model could then be used with Ministar S (SHEU, Germany) to produce the bleaching guards without heat damage.

Final decision on the way forward.

A base model and final fabrication of the bleaching guards could be manufactured following the experiences of the pilot study.

Appendix D: Fabrication of base models and bleaching guards

Following the pointers indicated in the pilot study, suitable base models and bleaching guards for the definitive study could be made.

Wax-up

Pink wax [Kemdent Modeling wax no: 4 (S.A.)] was melted into a plastic mould and left to set. Subsequently, pink wax was removed from the mould and formed the base of the base model (Figure D.1a). Teeth were randomly selected and a wax-up was done in an anatomical sequence, starting with four incisors, followed by two canines and five premolars. Each base model had a unique number and the teeth were numbered from one to eleven (Figure D.1 b). This unique number was repeated on the individual labelled bottles which stored the teeth in between bleach treatments. This ensured that correct specimen identification was ensured at all times.

Pack and cure process, cleaning and polishing of the base models.

Clear acrylic base models were produced in a dental laboratory through the pack and cure process. White dental stone, batch number: 10605237 (H&P Dental) and yellow dental stone, batch number: FCL 876 (H&P Dental) was used. The wax was boiled out and a clear acrylic, Vertex Acrylic Rapid Simplified (Vertex-Dental B.V.) was placed into the void left by the wax.

The acrylic was kept under a constant pressure of 100kp/cm^2 to force out excess material. After polymerization of the clear acrylic, the base models were cleaned and polished. A straight hand piece with various diameter round burs was used to remove the yellow stone from the base models (Figure D.1c, d). The base models were placed in an ultrasonic bath (Whaledent; BIOSONIC) to remove any yellow stone left in the base models.

A white linen polishing wheel (ZENETH, SA) with pumice and a white plastic polishing block (ZENETH, SA) were used to polish the base models.

The positions of the corresponding teeth were verified for a secure and precise fit on the base models (Figure D.1e). The roots of the teeth did not have the same anatomy and ensured that the teeth could only fit in its specific position on the specific base model. The meticulous labelling of the bottles (Section 3.2) and base models (Figure D.1b) prevented any confusion and switching of teeth.

A modified wooden frame (Figure D.1f) was made and an impression was taken of the base model holding the teeth. Yellow stone was poured into the impression [Alginate: Phase PLUS (Zermack, Italy)] and left to set (Figure D.1g).

Ministar S (SHEU, Germany) was used to place a one millimetre thick spacer over the yellow stone cast (Figure D.1h). The one millimetre spacer produces the necessary space for maximum contact between the bleaching agent and the teeth. The Opalescence Quick guard was two millimetre thick (Figure D.1i).

The bleaching guard was thicker than that which is usually used. The reason for this is that a thicker bleaching guard provides more stability, does not dislodge easily and provides better contact between teeth and the bleaching agent.

All the bleaching guards were made using Minister S (SHEU, Germany). A bleaching guard of three millimetres thick was used for the Control and Ozicure group (Figure D.1j and D.1k). The thickness of the bleaching guard was as according to Ozicure Oxygen Activator manufacturer's instructions. An inlet pipe was assembled into the guard in the area in front of the incisor teeth. Two outlet pipes are placed opposite the back premolars for ozone gas flow (Figure D.1k).

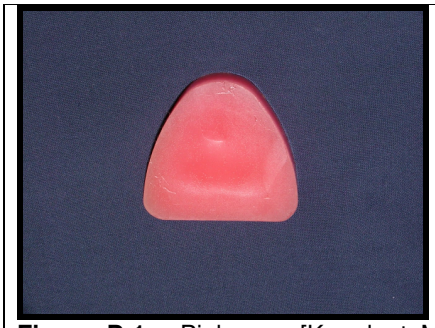


Figure D.1a. Pink wax [Kemdent Modeling Wax no: 4 (S.A.)] was used to produce the base models.



. **Figure D.1b.** A tooth wax up was done on the base models.



Figure D.1c. The teeth were removed and the pack and cure process followed to produce acrylic base models.



Figure D.1d. Acrylic base model was cleaned and polished.

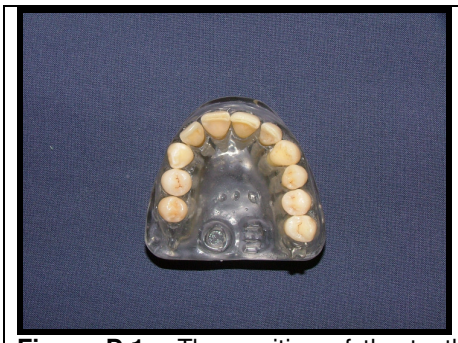


Figure D.1e. The position of the teeth was verified on the base model.

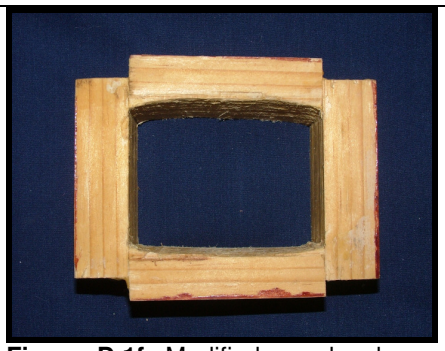


Figure D.1f. Modified wooden box used to take an impression with the teeth in position.



Figure D1g. Yellow stone mould poured and used to produce bleaching trays using Ministar S (SHEU, Germany).



Figure D.1h. Ministar S (SHEU, Germany) used for bleaching guard fabrication with a 1mm spacer.



Figure D.1i. The Opalescence bleaching guard (2mm thick Copyplast).



Figure D.1j. The control bleaching guard (3mm Copyplast).



Figure D.1k. The Ozicure bleaching guard (3mm thick Copyplast). Three small pipes were placed in the guard that would allow the sufficient flow of ozone gas.

Figure D.1a-k. Fabrication of base models and bleaching guards.

Appendix E: Tooth bleaching efficacy of the piloted system

Rationale

The efficiency of the base models and bleaching guards had to be tested, as well as establishing the number of bleaching cycles to obtain tooth lightening.

What is the problem?

Teeth would not be optimally bleached if the piloted system was ineffective. Study boundaries needed to be set on the frequency of bleach cycles undertaken in the study. It was already decided that all three anterior teeth (incisor, canine and premolar) would be used in the investigation.

Solving the problem.

Testing of the piloted bleaching system had to be evaluated with specific attention given to tooth colour change, frequency of bleach and tooth type. Six teeth were slotted into two base models of clear acrylic with the crowns protruding, each base model containing three teeth. Both base models had a specific position for every tooth (Figure E.1).

Ozicure Oxygen Activator

First phase: In the first phase the teeth are bleached with ozone produced by the Ozicure Oxygen Activator. The teeth in the base model were wetted with activated water and the bleaching guard was placed over the teeth and connected with the Ozicure Oxygen Activator. The total bleaching treatment time was 30 minutes.

Second phase: The teeth were bleached with Trèswhite (Ultradent, USA) strips. The second phase consisted of 30 minutes of tooth bleaching. The total bleaching time was one hour. The premolar in position one (Figure E.1) received one cycle of treatment (60 minutes). The incisor in position two received two cycles of treatment (120 minutes) and the canine in position three received three cycles of treatment (180 minutes).

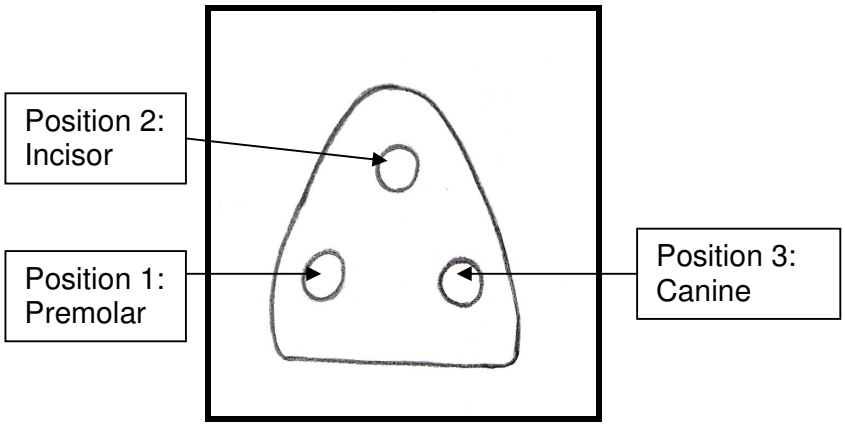


Figure E.1. Schematic illustration of the pilot study of the clear acrylic block with the three positions for the teeth.

Opalescence Quick.

The teeth were placed in position in the base model and covered with the bleaching guard containing the Opalescence Quick bleaching agent. The premolar in position one received one cycle of treatment (60 minutes). The incisor in position two received two cycles of treatment (120 minutes) and the canine in position three received three cycles of treatment (180 minutes).

Outcome of the options.

Opalescence Quick

During the treatment phase, the bleaching guards effectively brought the bleaching agents in contact with the teeth. This was confirmed by the gel adhering to the teeth after the bleach treatment. All the teeth lightened after the bleach treatment. The results for the Opalescence treatment are displayed in Table E.1.

Table E.1. Opalescence Quick pilot study bleaching results.

Tooth type	**1st bleaching**			**2nd bleaching**			**3rd bleaching**		
	Colour assessment times			**Colour assessment times**			**Colour assessment times**		
	Baseline	**1 hour**	**24 hours**	**Baseline**	**1 hour**	**24 hours**	**Baseline**	**1 hour**	**24 hours**
Premolar	3L2.5	2M2	1M2						
Incisor	3L2.5	2M2	2M2	2M2	2M2	2M2			
Canine	2M3	2M2	2M2	2M2	1M2	1M2	1M2	1M2	1M2

The first tooth colour change was seen after one hour of bleaching for all the teeth. The incisor tooth did not lighten further after the second bleaching treatment, in contrast the canine tooth progressively lightened with follow up bleaching treatments. The three tooth types responded differently to the bleaching treatment.

Ozicure Oxygen Activator

During the treatment phase, the bleaching guards effectively brought the bleaching agents in contact with the teeth. This was confirmed by the flow meter which was constantly monitored to ensure it regulated the ozone gas flow at 5l/min. The absence of leaks around the guards confirmed an optimum seal.

The results are presented in Table E.2. and show that the different types of teeth respond differently to the bleaching treatment. The incisor tooth showed tooth colour change only after the second bleaching. The canine tooth did show colour change after the first hour and second hour of tooth bleaching, but the overall tooth colour stayed the same after three bleaching treatments. The premolar tooth did not show any tooth colour change after bleaching.

Table E.2. Ozicure Oxygen Activator pilot study bleaching results.

Tooth type	1st bleaching			2nd bleaching			3rd bleaching		
	Colour assessment times			Colour assessment times			Colour assessment times		
	Baseline	1 hour	24 hours	Baseline	1 hour	24 hours	Baseline	1 hour	24 hours
Premolar	2M3	2M3	2M3						
Incisor	4M1	4M1	4M1	4M1	3M2	3M2			
Canine	2M3	2M2	2M3	2M3	2M2	2M3	2M3	2M3	2M3

Final decision on the way forward.

Both systems appeared to be effective since at least one tooth in each treatment showed colour lightening. It was felt that the differential tooth response to the bleach could be as a result of different tooth type and/or bleaching treatment and/or number of bleaches. Three bleach cycles were decided upon for the full investigation in light of the Opalescence Quick results.

Appendix F: Tooth surface effects of bleaching – mineral or tooth surface loss

Rationale

There is no consensus as to what effects bleaching have on tooth surfaces. One suggestion concerns enamel surface erosion in the form of loss of tooth material or tooth mineral.

What is the problem?

Bleached and unbleached tooth surfaces needed to be examined to establish if any morphological or mineral enamel changes followed tooth bleaching.

Options to solve the problem.

The scanning electron microscope was utilized to examine this aspect of the study. Two teeth from the pilot study, the premolar and incisor teeth from the Opalescence Quick group were embedded in resin and sectioned horizontally through the tooth crown bisecting the nail varnish.

The two sectioned parts were re-embedded in resin and polished and coated with carbon in an Edwards Vacuum Coating Unit, model E12E (Edwards Vacuum Ltd, Crawley, UK) for conducting purposes. Viewing took place using a JEOL JSM-840 SEM (JEOL, Akishima, Japan) at 10kV. Sub-surface enamel elemental analysis was undertaken in two areas of the polished specimen using a LINK AN10000 energy dispersive x-ray analyzer (EDX) [Oxford Instruments, High Wycombe, UK]. Analysis conditions were 10kV, a live counting time of 100 seconds and a count rate of 2000 counts per second. The areas examined were in the area immediately below the exposed tooth surface and immediately below the varnish-covered area. The Ca:P ratio was found to be similar in both cases. No apparent visual difference was found between the tooth surfaces covered with nail varnish and the exposed tooth surfaces.

Final decision on the way forward.

It was felt the resolution and analysis powers of the instrument were not sensitive enough to detect any mineral or enamel surface loss. Consequently, a superior imaging resolution SEM was utilized in the latter part of the study and this is described in the methods and results sections of the research report.

Appendix G Tooth surface effects of bleaching – surface roughness

Rationale

There is no consensus as to what effects bleaching have on tooth surfaces. One of the indications is that surface roughness changes could occur following bleaching.

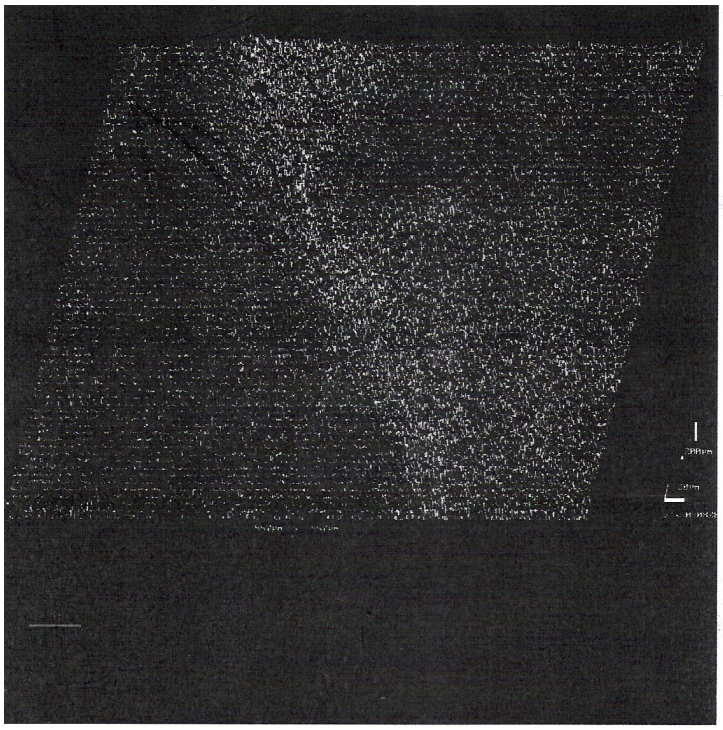
What is the problem?

A suitable means for assessing surface roughness needed to be established.

Options to solve the problem.

Confocal Laser Scanning Microscope

A Zeiss LSM 410 Confocal Laser Scanning Microscope (Carl Zeiss Jena GmbH, Jena, Germany), was first used to see if it would be able to produce the necessary resolution for tooth surface roughness. An incisor tooth was used for the test, but due to the magnitude of the curvature of the tooth, the surface roughness of the incisor tooth could not be measured. The resolution of the instrument was found to be unsatisfactory for the purposes of the study.



557µm x 55µm

Micro Systems
Zeis LSM

LSAM Ref11
Control = 612
Bright = 8810
Pinhole = 23

Zoom = 2.3
Lens = 1
Magn = 10 x 10.25

X scale = 0.6625µm
Y scale = 8µm

Laser 488
Alten = 1
t = 1; 8,16s
Bandw = 0
D = 1
FT 488/568

X = 0µm
Y = 0µm
Z = 2443.4µmE

Figure G.1. Confocal laser scanning microscope photograph revealing unsatisfactory resolution.

Atomic Force Microscope

Following further investigation the Veeco di CP-II AFM (Veeco, Santa Barbara, California, USA) became the microscope of choice to establish the effect of bleaching on surface roughness of bleached enamel.

Another problem that came out from the pilot studies using the AFM.

The scanning tip of AFM can scan around a curvature, but only within limits. The enamel tooth surface to be examined, had to be perpendicular to the scanning tip and stable during the scanning process. Three mounting methods were explored to obtain the best stability.

1. Standard double sided tape - Double sided tape was found not capable of keeping the tooth sufficiently stable for AFM purposes.

2. Superglue - Superglue was next tested for suitability as a mounting medium. Superglue had to be placed in significant quantities to keep the teeth in position and a hairdryer had to be used to speed up the setting time. The superglue was also found to be incapable of keeping the teeth stable during the scanning process and this method was also discarded.

3. Pratley Quickset Clear (Pratley®) - The instructions of this adhesive indicate that the set time is approximately 20 minutes. This was found not to be the case, as the teeth were not secure after this time. The samples thus were placed in an oven for four hours at 40℃ to ensure optimal curing of the Pratley Quickset Clear (Pratley®). This procedure ensured a rigid, secure mounting of the teeth to the steel disc.

Piloting the surface effects on the tooth enamel.

A maxillary central incisor tooth was use to pilot the effect of the bleach (Opalescence Quick) on surface roughness. The incisor tooth was covered half with clear nail varnish and the other half of the tooth enamel was left exposed (Figure G.2). The tooth received 3 cycles of one-hour treatments each with Opalescence Quick.

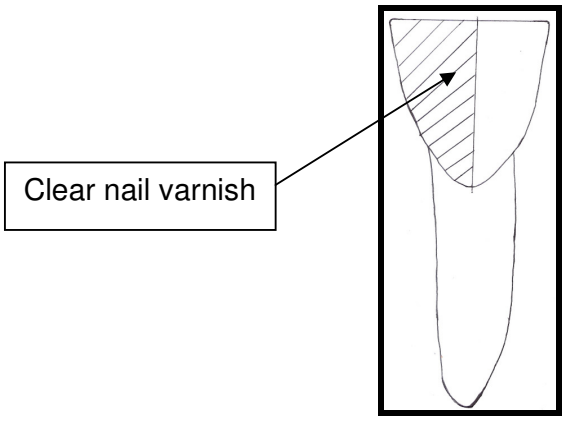


Figure G.2. Half of the maxillary incisor tooth was covered with clear nail varnish to create a control to which the surface roughness could be compared.

After the bleaching procedures, the clear nail varnish was removed with acetone: this surface served as the control (unbleached surface). The root mean square (rms) value of the surface roughness was measured on bleached and unbleached surfaces.

The incisor tooth gave more reliable and constant readings since its curvature was not as profound as for the canine and premolar teeth. In addition, the surface roughness of premolar teeth was considerable and sometimes too extreme to measure. The samples were scanned over squares of side length of one, 10 and 50 micrometres. Ten micrometres was determined experimentally to be the best suitable scanning size for the necessary detail that was required. Twenty scans were made, 10 on the exposed enamel surface and 10 on the varnished covered enamel surface.

Unbleached surface

The histogram of the surface roughness from each pixel in the whole of the scanned region in Figure G.3 resulted in a skewed Gaussian distribution, Figure G.4 (the red and blue vertical lines have no meaning) showing that the roughness is dominated by roughness smaller than the mean value of 154nm.

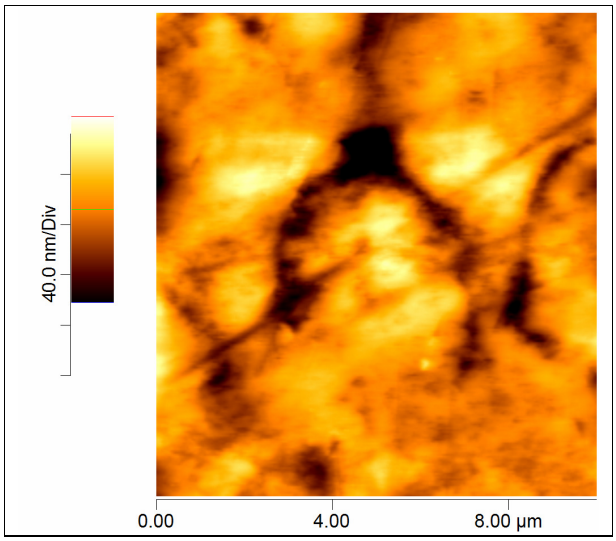


Figure G.3. AFM photograph of the non-bleached tooth surface. Scanning was done over a square of side length of 10µm. The average surface roughness was 18.6nm.

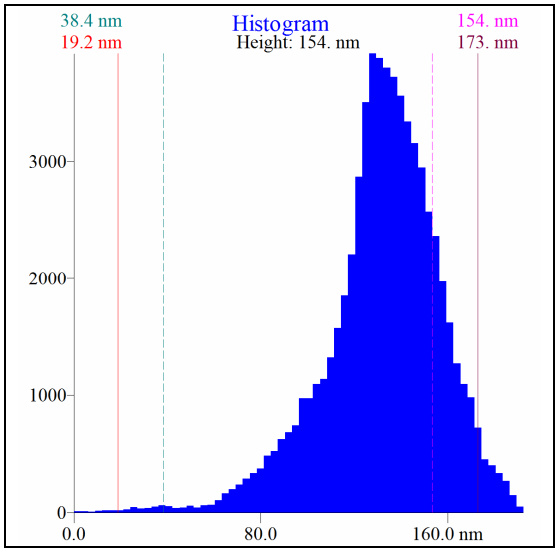


Figure G.4. Histogram of the surface roughness showing a skewed left Gaussian distribution of the non-bleached tooth surface area.

Bleached surface

Figure G.5 is typical AFM micrograph of the bleached tooth surface. The histogram of the surface roughness exhibits a bell shape or Gaussian distribution, meaning that the roughness is symmetrical about the peak roughness of 111nm for this sample (Figure G.6).

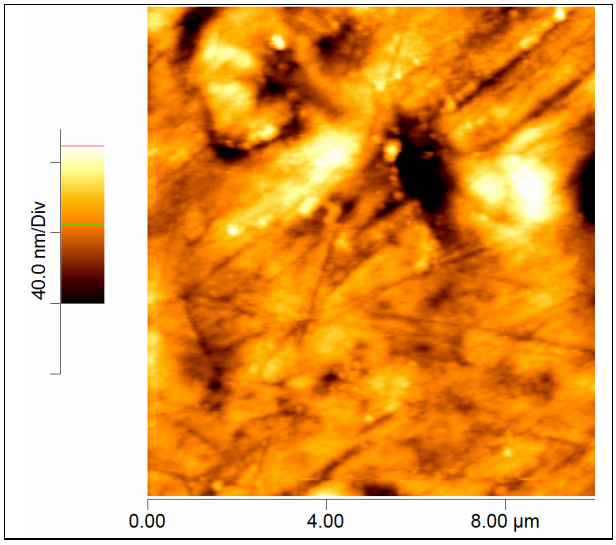


Figure G.5. AFM photograph of the bleached tooth surface. Scanning was done over a square side length of 10µm in the X and Y direction. The average surface roughness was 10.8nm.

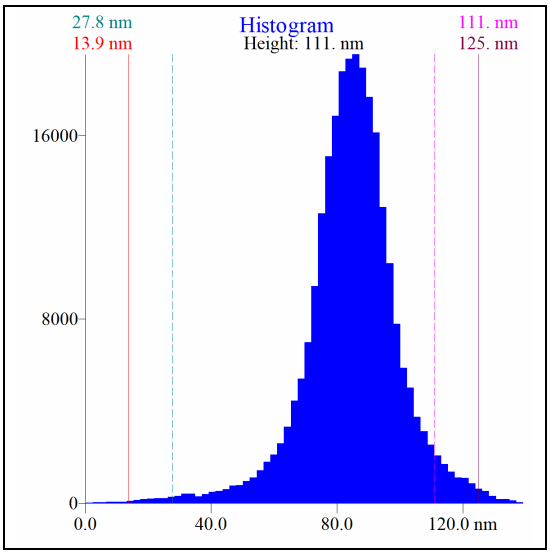


Figure G.6. Histogram showing a Bell-shape or a symmetrical Gaussian distribution of the bleached tooth surface area.

Final decision on the way forward.

The AFM was subsequently used for the surface roughness studies undertaken for the research report. Pratley Quickset Clear (Pratley®) was always used to mount the specimens on the steel specimen disc. The side length of the scanning square was fixed at 10µm since it was determined to be the best suitable scanning size for the necessary detail that was required.