Effect of bleaching agents on enamel surface microroughness, micromorphology and microhardness: comparison of carbamide peroxide and hydrogen peroxide - an in vitro study
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إلى والدي الأعزاء
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1 Introduction

Bright and white are considered as a dream for every patient who has a problem with his tooth color. Smile is an important factor for communication between people. One of the frequently asked questions of patients in dental praxis after controlling the pain is: “How can I have brighter teeth?” Many considerations must be taken before answering this question. What does discoloration of teeth cause? How intensive is it? Which teeth are involved in this problem? Tooth Discoloration is a common aesthetic problem. Teeth staining may be due to external pigments, such as tobacco, coffee, tea or internal factors, such as loss of tooth vitality, systemic diseases or drugs (Hattab *et al.* 1999; Leard & Addy 1997; Plotino *et al.* 2008; Watts & Addy 2001). Most of the external stains can be removed with professional cleaning or brushing of the teeth. Internal stains are difficult to remove by brushing or cleaning because the pigments penetrate into the structure of the teeth (Matthews 2001).

The quest for whiter teeth can include number of treatment options, from bleaching to microabrasion, bonding, veneers or crowns. Currently the most conservative treatment which requires no removal of tooth substance is bleaching. In some cases, more than one technique may be used to achieve good results (Suzuki *et al.* 1982). Overall, tooth bleaching is the first choice concerning conservative aspects because no hard tissue has to be removed from the teeth (Selim 1994). Tooth bleaching has become one of the most popular procedures in dental praxis (Kihn 2007). Many whitening techniques have been developed in terms of application and concentration of bleaching agents. Before bleaching one question should be answered: which effects do bleaching materials have on the enamel surface?

So far, there is no agreement about the morphologic alterations of enamel surface or microhardness after bleaching procedures (Borges *et al.* 2009; de
Magalhaes et al. 2009; Dutra et al. 2009; Ren et al. 2009). This controversy may be due to different experimental protocols used to test bleaching agents, e.g. differences in commercial components of products tested, the pH of these agents or time of bleaching. Sulieman et al. (2004) reported that hydrogen peroxide (HP) had no deleterious effects on enamel, but the pH of the bleaching materials might cause adverse effects during bleaching procedure. Examination of enamel surfaces after bleaching with 30% carbamide peroxide (CP) by atomic force microscopy (Mahringer et al. 2009) demonstrated minimal increase of enamel roughness (from 19±4 nm to 33±5 nm). This increase of roughness happened during the first hour of bleaching, while no further change was observed in the next 6 hours of bleaching. The authors suggested that the partial lysis of the matrix proteins of tooth enamel might cause these changes. Faraoni-Romano et al. (2008) showed no alteration of bovine enamel after bleaching with low (10% CP) and relative high (38% HP) concentrated bleaching agents concerning microhardness and surface roughness. Also tooth bleaching during 21 days with 10% CP and 7.5% HP (Sasaki et al. 2009) demonstrated no significant changes concerning micromorphology and microhardness of enamel after the treatment. However, an increase concerning microhardness of enamel was recorded after storing the specimens for 14 days in artificial saliva after the end of the treatment.

Therefore, the aim of this study was to investigate the influence of bleaching procedures using CP or direct application of HP and the effect of low versus high concentrations of bleaching agents on the micromorphology, microroughness and microhardness of enamel surface using laser profilometer, scanning electron microscope and Knoop-hardness tester.
2 Literature review

2.1 Dental enamel

Enamel is the hardest tissue of the human body. The main functions of enamel are to protect the softer underlying dentin and as a surface substance for chewing and crushing food. It is translucent and ranges from yellow to grey-white. This range is due to a varying thickness of the enamel layer. It consists of 96% minerals and 4% organic matrix. The thickness of enamel varies on the surface of teeth. It is thicker at the cusps (about 2.5 mm) and the thickness of enamel decreases gradually from cusps or incisal edges to cemento-enamel junction (Chandra Satish 2007; Ten Cate 1990). The basic unit of the enamel is the enamel rod. The number of these rods is constant, but the enamel volume increases from the cemento-enamel junction to the outer surface. This increase of the volume can be explained by an increasing diameter of these rods (Skobe & Stern 1980). The secretion of a complex mixture of proteins by ameloblasts initiates the enamel biosynthesis (Paine et al. 2000; Ravindranath et al. 2001). The enamel secretion begin from the dentin-o-enamel junction up to the enamel surface (Radlanski et al. 2001). The biomineralization of this matrix is achieved by depositing mineral ions in the matrix to form hydroxyapatite crystals. As a result, it is the hardest mineralized tissue in the body (do Espirito Santo et al. 2006). The apatite crystals lie together in roughly hexagonal form as seen in cross sections. These crystals appear as small rods in the side view. A special characteristic feature of the enamel crystals are their size in comparison to other biological crystals. They are 160 nm long, 40-70 nm broad and 26 nm high. However, the shape and size of enamel crystals can differ according to the degree of enamel maturation, or localization in the enamel coat. About 100 enamel crystals form enamel prisms. These rods extend from the enamel dentin border to the enamel surface (Hellwig et al. 2009). The main proteins of the organic matrix are amelogenin, tuftelin and ameloblastin (Paine et al. 1998; Paine
& Snead 1997). The bulk of organic part, which consists of tyrosine-rich amelogenin polypeptide (TRAP), binds tightly to the hydroxyapatite crystals (Ten Cate 1990). The daily rate of the production of human enamel is about 3.5 µm. It consists of tightly packed hydroxyapatite crystals organized into a pattern of prisms and interprisms (Risnes 1998). There is correlation between magnesium and carbonate and degraded values of the enamel density (Hellwig et al. 2009). Regions with an increased magnesium concentration (near dentin horns and directly under the central fissures) demonstrate lower density than more mineralized zones, such as buccal and lingual surfaces of the crown. Calcium and phosphorus exist in the tooth in a proportion of 1:1.2 in small crystals of apatite (Ca_{10-x}P_{06-x}) \times X_2 \times H_2O. The spaces between the prisms and the crystallites are filled with water and organic material (protein and lipids) (Ten Cate & Featherstone 1991). These spaces form the diffusion pathways for acids, mineral components and fluoride ions. The hardness of enamel varies over the external surface of tooth according to the location of area. The hardness also decreases from outer surface of the enamel to its inner surface (Satish Chandra 2007).

2.2 Enamel erosion, abrasion, attrition

Erosion is defined as painless irreversible loss of dental hard tissue due to chemical dissolution caused by acids of any origin except the bacterial activity (Cairns et al. 2002). The enamel erosion caused by acids from bacteria is called dental caries (Larsen & Nyvad 1999). Abrasion is defined as mechanical wear from e.g. inappropriate brushing habits together with abrasive dentifrices (Eccles 1982). Capability of materials to erode enamel depends on the pH value of this substance. The critical pH value for the dissolution of tooth minerals of enamel is pH<5.5 (Cairns et al. 2002). Any material that comes in contact with teeth and has acidic property can lead to defects of the enamel surface. The severity of this defect depends on many factors such as pH value of the erosive
agent, duration of contact with enamel and its buffering effect. Soft drinks, diet systems and some dental procedures (such as tooth bleaching) have been discussed concerning enamel erosion (Pretty et al. 2005; Ren et al. 2009; Ushigome et al. 2009). There are extrinsic and intrinsic factors for tooth erosion. Intrinsic factors are involuntary gastrointestinal disturbances such as gastro-esophageal reflux disease (GERD). Extrinsic tooth erosion is caused by environmental factors, medicaments, lifestyle and diet (Shipley et al. 2005). Since 1908 Black suggested eight factors causing dental erosion (Wood et al. 2008):

- Malformation of teeth
- Friction from an abrasive tooth powder
- Action of an unknown acid
- Secretion from a diseased salivary gland
- Physiological resorption, as with deciduous teeth
- Acid associated with gouty diarethis
- Action of alkaline fluids on calcium salts
- Action of enzymes released by microorganisms

However, As long ago as 1908, GV Black mentioned by discussing the problematic etiology “erosions”, and stated that “our information regarding erosion is far from complete and much time may elapse before its investigation will give satisfactory results” (Wood et al. 2008).

### 2.3 Surface roughness measurement

Surface roughness measurement is used to determine the texture of a surface. It is quantified by vertical deviations from its ideal form. If these deviations are large, the surface is rough. If they are small the surface is smooth. Roughness can be an important parameter in determining how the enamel surface will interact with the applied materials. Relevant changes of roughness values before
and after application of tested materials can provide important information about the materials. It is measured using contact or non-contact methods. A contact method includes stylus that is moved vertically in contact with a sample and then moved laterally across the sample for a specified distance and specified contact force. By using a stylus the measured surface may be scratched, which in turn could seriously affect the roughness values if the measuring surface is very smooth. Another problem is that the stylus may be too blunt to reach the bottom of deep valleys in the surface profile and it may round the tips of sharp peaks (Fig.1). A non-contact method, such as laser profilometer will be preferable in such cases. The surface profile will be scanned with a light source instead of the classical probe stylus (Kocher et al. 2002).

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**Fig.1:** The path of a probe stylus is drawn (red line) over a real surface. The illustration shows the mechanical filtering effect of the stylus dimensions. The stylus may be too blunt to reach the bottom of deep valleys in the surface profile and it may round the tips of sharp peaks (source: www.wikipedia.org. Permission is granted to copy, distribute and/or modify this document under the terms of the GNU Free Documentation License)
2.3.1 Roughness parameters

There are different parameters to describe the roughness of the surface. Each of the roughness parameters is calculated using a formula for describing the surface. The International Standards Organization (ISO) unified these parameters, so that these parameters can be measured according to the ISO or DIN (Deutsche Institut für Normung).

Roughness average (Ra)

Ra (DIN 4768, ISO/DIS 4287/1) is the most common parameter used to describe the surface profile. Ra is the arithmetic mean of absolute values of the surface departures from the mean plane within the sampling area (Kocher et al. 2002). It reflects average condition of the roughness profile. Ra is the area between a roughness profile and its mean as demonstrated in (Fig.2).

\[ R_a = \frac{1}{n} \sum_{i=1}^{n} |y_i| \]

Fig.2: Ra is the arithmetic average of the absolute values of the roughness profile ordinates. \( i \) = sampling length, \( n \) = number of sampling in the evaluated length, \( y_i \) = is the vertical distance from the mean line.

Roughness average in Z dimension (RzDIN)

RzDIN (according to DIN 4768/1) is the arithmetic mean of five single rough depths (Fig3). Rz is more sensitive to the changes on the surface profile than Ra because maximum profile heights and not averages are being examined.
Maximal roughness depth Rt-Ry

Rt-Ry (according to DIN 4762/1E) is the vertical distance between the highest and deepest point of the scanned area.

2.4 History of tooth bleaching

White teeth were a sign of the health. Romans had used urine for dental cleaning to make the teeth whiter (Schmidseder 1998). In the Middle Ages different recipes with nitric acid were used. In 1918 Abbot combined heat, light and hydrogen peroxide for bleaching of discolored teeth (Feinmann 1988). This method was the precursor of many current bleaching technologies. The main factor for bleaching of teeth is an oxidation reaction. Many oxidizing agents were used to accomplish tooth whitening. The first dental report published for tooth bleaching using oxalic acid was in 1877 by Chapple (Araujo et al. 2003). Hydrogen peroxide as bleaching agent in dentistry has been described by Harlan in 1884 (Hegedus et al. 1999). In 1916 Walter has used 18% HCl for bleaching fluorosed teeth (Tom 1992). The first description of the “walking bleach technique” with a mixture of sodium perborate and distilled water was
mentioned in a congress report by Marsh and published by Salvas in 1938 (Plotino et al. 2008). A successful home bleaching technique using hydrogen peroxide was first described by Klusmier in 1968 (Haywood 1990). The so-called “at-home” night-guard vital tooth bleaching has first described in 1989. This technique was described by using 10% carbamide peroxide, placed in custom fitted trays by the patient and worn at night (Haywood & Heymann 1989). Due to high success rates of bleaching teeth in many cases, bleaching techniques nowadays have been introduced as a treatment for many kinds of discolorations. Although there is a huge number of bleaching products, whitening techniques base on the oxidation reaction through the active bleaching component of the product.

2.5 Mechanism of tooth bleaching

Tooth staining can be removed by certain chemical reactions. The actual mechanism of bleaching is not yet clarified in detail (Sulieman 2004). Researchs have shown how easily hydrogen peroxide, passes through the enamel and dentin due to its low molecular weight (Bowles & Ugwuneri 1987; Jiang et al. 2008). It has been suspected that free radicals, released from H₂O₂, attack the organic components of the tooth and pigmented molecules. This attack will cause a destruction of the long organic chains into colorless short chains by an oxidizing reaction creating a whitening effect (Wiegand et al. 2005).

The tooth color is determined mainly by dentin, whereas enamel plays only a minor role through scattering wavelengths in the blue range (ten Bosch & Coops 1995). The ability of bleaching agents to pass through the enamel to dentin to make it lighter is another factor making tooth whiter after bleaching of teeth. Nathoo et al. (2003) revealed that application of either a 25% carbamide peroxide gel or a 8.7% hydrogen peroxide gel once a day gave a statistically significant tooth shade lightening after 3 weeks (Nathoo et al. 2003). This study
demonstrated no statistically significant differences in the bleaching effect between hydrogen peroxide vs. carbamide peroxide.

2.6 Tooth discoloration

Tooth discoloration is any change in the hue, chroma, value or translucency of a tooth due to any cause. Restorative filling materials, drugs (both topical and systemic), pulpal necrosis or hemorrhage are important factors that are responsible for discolorations. There are external and internal teeth discolorations:

2.6.1 External tooth discoloration

Extrinsic stains are caused by extrinsic agents and are located on the outer surface of the teeth (Hattab et al. 1999). They occur after tooth eruption. The evaluation of discoloration depends on the intensity, distribution and color change (Eriksen & Nordbo 1978). Extrinsic discoloration appears when external chromogens accumulate on the tooth surface or within the pellicle layer (Sulieman et al. 2003). The Nathoo classification system of extrinsic dental stains (Nathoo 1997) describes three categories of teeth discoloration as follows:

Nathoo type 1 (N1): Direct dental stain

In this type, the chromogen binds to the tooth surface and leads to discolorations. Chromogens can be coffee, tea, red wine, tobacco or berries. The color of chromogen and the discoloration are identical (i.e. brown discoloration of teeth due of smoking).

Nathoo type 2 (N2): adhesive direct dental stain

The chromogen binds to the dental surface. Nevertheless, the color of the Chromogen and the discoloration are not identical. The discoloration in this
type is caused by metal ions and leads to a stronger adherence in comparison to type N1. Removal of tooth discoloration of type N2 is more difficult than of type N1. (i.e. Fe-III-chloride or silver nitrate leading to gray or black discoloration).

**Nathoo type 3 (N3): indirect dental stain**

A colorless material or prechromogen binds to the tooth and undergoes a chemical reaction to cause a stain. Examples are stannous fluoride and chlorhexidine.

**Causes of external tooth discoloration**

**Chlorhexidine (CHX)**

CHX is used in different forms in dentistry such as varnish (Attin et al. 2008) or chips for local antibacterial therapy in the pathological periodontal pockets (Jeffcoat et al. 2000). CHX in form of gel, spray or mouthwash is described as effective agent in compensation with mechanical plaque control, especially during periodontal therapy (Addy & Moran 1997; Quirynen et al. 2001; Sekino et al. 2003). The most common side effect when using CHX in the oral cavity is staining (Carpenter et al. 2005; Eriksen et al. 1983; Lee & Powers 2006; Solheim et al. 1980), and sometimes disturbance of taste (Gurgan et al. 2006). It has been suggested that CHX may react with components of the diet to produce the staining seen clinically (Prayitno 1979). The other study stated that chlorhexidine molecules themselves may be involved in the development of stained products (Heyden 1973).

**Food, drinks, smoking**

Food with color components such as blueberries, coffee, tea or red wine can cause tooth discoloration (Eriksen & Nordbo 1978; Proctor et al. 2005). Smoking was proven to stain teeth. The severity of discoloration depends on the amount
of cigarette consume (Ness et al. 1977). Food habits, such as chewing tobacco and spices, can stain the teeth with a black color (Tayanin & Bratthall 2006). Organic molecules containing a certain atomic configuration, namely -N=C-C=N- have been known to react with certain metal ions such as ferrous, cuprous and cobaltous resulting in colored complex species (Dennon et al. 1970).

**Bacteria and discoloration**

The activity of chromogenic bacteria in plaque may produce yellow, green, or orange color. Black stains of teeth were described by Theilade & Pang (1987). Black stains can reform after removing it (Eriksen & Nordbo 1978). It has been suggested that black stains are caused by microorganisms in the saliva and are located parallel to the gingiva (Paredes Gallardo & Paredes Cencillo 2005). However, Reid et al. (1977) confirmed the theory described by Theilade et al. (1987) that black extrinsic tooth stains are a form of dental plaque (Reid et al. 1977). The stain contains a black insoluble ferric compound, probably ferric sulphide. The compound is thought to arise from the interaction of hydrogen sulfide produced by bacteria in the periodontal environment and iron in saliva or gingival fluid.

**2.6.2 Internal tooth discoloration**

Chromogens deposit within the tooth structure, usually in the dentine, and are often of systemic or pulpal origin (Plotino et al. 2008). There are two divisions groups of intrinsic stains: pre- and post-eruptive intrinsic stains.

**Pre-eruptive intrinsic stains**

Some drugs have a potential to induce changes in the teeth, leading to internal tooth discoloration. These effects may cause physical injury to the tooth structure or alteration in sensitivity of the dentin, cementum or enamel (Tredwin et al. 2005).
Antibiotics
Tetracycline and Erythromycin are commonly used systemic antibiotics. Because tetracycline can cause enamel dysplasia and tooth discoloration it should not be used during pregnancy or for children under the age of 12 years. Tetracycline causes internal tooth discoloration when used during tooth development (Ritter 2005). In 1963, the United States of Food and Drug Administration issued a warning about the use of such antibiotics in pregnancy and childhood (Hattab et al. 1999). There are basically four types: oxytetracycline, chlortetracycline, demethylchlortetracycline and tetracycline. The intensity of staining varies depending on the type and concentration of the antibiotics, as well as the duration of the drug intake (Arens et al. 1972; Billings et al. 2004; Ritter 2005). The color varied between yellow to gray-brown (Billings et al. 2004).

Dental fluorosis
Fluoride increases tooth resistance against caries (Rasines 2010; Topping & Assaf 2005). This prevention against decay depends on the fluoride level and source. Discoloration of teeth occurs when the daily intake of the fluoride ion is high during enamel formation and maturation. An average daily intake of < 0.04 mg F/kg carries a low risk for fluorosis (Hong et al. 2006). A daily intake of 0.04–0.06 mg F/kg generally produces a significant increased risk for fluorosis, and a daily intake of 0.06 mg F/kg is associated with a high risk for fluorosis (Hong et al. 2006). The chronic toxic concentrations affect the function of ameloblasts during formation of the enamel matrix (Hellwig et al. 2009).
There are different sources of fluoride such as water, fluoride drops, tablets and tooth paste (Tredwin et al. 2005). Similar to tetracycline exposure, the extent and severity of clinical findings are correlated to the dose and duration of fluoride exposure during the tooth development. Dental fluorosis can be treated by
bleaching, micro-abrasion, veneers or crowns. The severity of the fluorosis determines the choice of one or more of these treatments (Akpata 2001).

2.6.2.1 Post-eruptive intrinsic stains

Trauma and pulp necrosis
Loss of vitality after trauma can cause tooth discoloration (Wray & Welbury 2001). Decomposition of necrotic pulp tissues after death of the pulp and hemorrhage into the pulp chamber lead to this discoloration (Kawamoto & Tsujimoto 2004).

Caries
Primary and secondary caries can change the tooth color from yellow-brown to black due to bacterial activity in the lesion. Dental caries is a process where bacterial acids destroy hard dental tissue (Kleter 1998). As a result of dental tissue destruction, the light reflection and absorption process, which determine the tooth color, is affected. Initial lesions appear as opaque white spots and arrested lesions as brown spots. Treatment of caries improves the tooth color and result in acceptable aesthetic results. Bleaching teeth should be applied only on caries free teeth.

Dental materials
Partovi et al. suggested that endodontic sealers (AH26, endofill, tubliseal, zinc oxide, eugenol) can cause tooth discoloration after the application that also might increase over time (Partovi et al. 2006). The most affected area is the cervical third of the crown. According to their study the main cause of this discoloration are sealer remnants in the pulp chamber. Removing the smear layer during the treatment could increase the discoloration, due to opening the dentin tubules in the pulp chamber (Parsons et al. 2001). Staining of enamel after
orthodontic treatment has also been reported (Hodges et al. 2000). This discoloration may be a result of metal ions released by corrosion of metallic attachments.

A marked dark discolouration of dentine can be seen after the removal of amalgam. The penetration of corrosion particles from overlying amalgam into dentin is responsible for such staining (Scholtanus et al. 2009). However, concerning the relationship between amalgams and bleaching teeth, the replacement of amalgam filling should be considered and decided prior to bleaching (Deliperi 2007).

Mineral trioxide aggregate (MTA) has been successfully used as sealer in case of tooth perforation (Mamaladze & Sanodze 2008; Pace et al. 2008) or direct pulp capping (Asgary et al. 2008). Despite the favorable biological and mechanical properties of white MTA, tooth discoloration may occur after usage of MTA (Jacobovitz & de Lima 2008).

**Age of the patient**
Discoloration of teeth increases with age (Eriksen & Nordbo 1978). The upper layer of the enamel begins to get worn away over time. Gradually, the inner layer, which is yellowish, becomes revealed. There are factors that can affect the age-related discoloration, such as the genetics. Some people have brighter or yellowish teeth genetically. On the other hand, the continuously formation of dentin to protect the pulp and obliteration the dentin tubules is another factor for teeth discoloration with age. Age-conditioned dental discolorations can be treated by bleaching of teeth (Joiner 2006).
2.7 Treatment of tooth discoloration

2.7.1 Micro-abrasion

Micro-abrasion is a conservative procedure used to remove local internal stains on the enamel surface such as white spots or mild fluorosis (Croll 1990). In the micro-abrasion techniques 18% hydrochloric acid or 35% phosphoric acid mixed with pumice is used to achieve a maximum removal of 100 µm of enamel. The technique was described by Wray and Welbury (2001).

2.7.2 Veneers

Veneers are thin crusts of ceramic or composite those are bounded onto the front side of the teeth to improve their aesthetic appearance. The aesthetic rehabilitation of anterior teeth with veneers is one of the minimal invasive aesthetic solutions (Lambert 2006). In some cases, abnormalities of enamel can be treated with veneers, whereas bleaching technique treats just the tooth color. There are several types of veneers used commonly. Direct resin-based composite veneers or ceramic veneers are common choices for such a treatment.

Ceramic veneers can be used when bleaching teeth fails. In comparison to composite veneers, the aesthetic result of ceramic veneers appears more natural and resists staining more than composite veneers.

2.7.3 Tooth bleaching

Bleaching teeth can be performed on vital and on non-vital teeth. Teeth can be bleached by internal and external bleaching techniques. The internal bleaching, which also known as "walking bleach technique", is limited to the treatment of non-vital teeth. External bleaching techniques can be used on vital and non-vital teeth.
Concerning duration of bleaching results, the dentist needs to discuss with the patient that the results might be stable one to three years, and then the treatment will need to be repeated. Also, it cannot be determined prior to treatment how the teeth will respond. Some cases may take as many as four to six treatments to get the desired results with respect to the technique concerning concentration of whitening agent and time of exposure.

2.7.3.1 Internal bleaching

“Walking bleach technique” developed by Nutting and Poe in 1963 (Kawamoto & Tsujimoto 2004). They used a paste consisting of 30% H₂O₂ with sodium perborate in the pulp chamber. The mixture is applied in the pulp chamber for three to five days after sealing the cavity with a temporary filling. This process can be repeated several times if necessary. Rotstein et al. (1991) recommended the usage of sodium perborate in combination with water rather than with hydrogen peroxide to reduce the risk of post-bleaching external root resorption (Rotstein et al. 1991). They demonstrated the same results after 3 and 7 days by using water or 30% hydrogen peroxide as a mixture solution for this technique. Walking bleach technique has been described by many studies as a successful technique for intra-coronal bleaching (Lai et al. 2003; Nutting & Poe 1967; Rotstein et al. 1993). Rubber dam is applied to protect the adjacent structures. The access of the cavity should be shaped in a way that remnants of restorative materials, root-filling materials, and necrotic pulp tissue are completely removed. Some studies recommended additional cleaning of the pulp cavity with sodium hypochlorite (Attin et al. 2003). Other studies suggested conditioning the dentin surface in the pulp chamber with 37% orthophosphoric acid to remove the smear layer and to open the dentinal tubules (Plotino et al. 2008). This promotes the penetration of the bleaching agent deeply into the tubules and therefore may increase its effectiveness. However, some studies reported the risk of invasive cervical resorption in relation to intra-coronal
bleaching (Liebenberg 2007; Marin 2006). The main theory for the cause of this resorption is that the by-products of bleaching process diffuse from the pulp chamber to the outer root surface. This diffusion may induce trauma or damage of the cementum and/or periodontal ligament. Madison and Walton suggested these factors for possible resorption after tooth bleaching (Madison & Walton 1990). The other theory for tooth resorption after bleaching is that the peroxide from the whitening agent would leak through the dentinal tubules to the cervical area of the tooth, initiating an inflammatory resorption process (Ritter et al. 2002).

2.7.3.2 External bleaching

Home Bleaching

Home bleaching is known as night-guard vital bleaching (NGVB). It is the most common used technique because of its easiness of application, low cost and wide acceptance by patients (Haywood 2000). Home bleaching technique was firstly introduced in 1989 by Haywood and Heymann (Haywood & Heymann 1989). Home bleaching kits are available for daytime or nighttime. In this technique patients use carbamide peroxide gel in a custom-fitted soft plastic tray overnight or for a few hours during the day. The time of application depends on the reaction of a tooth color and a cause of discoloration. The bleaching tray can be used between 6 to 8 hours up to 6 weeks (dos Santos Medeiros & de Lima 2008). Clinical studies (Gegauff et al. 1993; Meireles et al. 2008; Swift et al. 1999) have shown that at-home application of 10% carbamide-peroxide-based products effectively whiten the teeth. Some studies reported that NGVB had high longevity (Leonard et al. 2001; Ritter et al. 2002). Leonard showed acceptable bleaching results after NGVB (Leonard 2003). They demonstrated that 43% of the bleaching patients were satisfied with the treatment results after 10 years of the treatment. Most clinical studies (Haywood et al. 1994; Tam 1999) show that tooth
sensitivity is the most prevalent side effect associated with this technique, followed by gingival irritation. However, these effects are temporary and disappeared at the end of the treatment (Leonard et al. 2001).

**In office bleaching**

This bleaching technique requires the use of high concentration of hydrogen peroxide (30-40 %) and direct monitoring by the dentist. In-office bleaching procedures can be an alternative to home-bleaching and can be useful especially in case of very severe discolorations, single tooth treatment or if a rapid treatment is needed. In-office bleaching technique can be used also as initial step before home bleaching to accelerate the bleaching results (Efeoglu et al. 2007). The soft tissue in the mouth should be protected during the treatment because of the high concentration of hydrogen peroxide (Walsh 2000). There is no rule in determination the frequency of treatment to obtain the favorable results. The severity, cause and depth of discoloration influence the treatment strategy. Generally one time application of in-office bleaching seems to be inadequate (Dahl & Pallesen 2003).

**Over-the-counter bleaching**

The newest systems that aim to bleach the teeth are bleaching kits that can be sold directly to consumers without the control of dentists (Kugel 2003). Over-the-counter (OTC) bleaching products are a low-cost alternative to bleach discolored teeth without dentist supervision (Demarco et al. 2009). There are variable choices of over-the-counter (OTC) peroxide-based products. Many of these products -especially those advertised on television or over the internet- are not safe, reliable or effective. It may be acidic and swallowed easily with potential of damaging the tooth enamel. Some OTC products use unproven means to deliver the whitening substance such as “boil-and-bite” mouth trays that can be uncomfortable. Some of these products have not undergone the
vigorous testing of professionally dispensed products. However, other OTC peroxide-based products such as polyethylene whitening strips contain hydrogen peroxide gels reported as safe method for OTC bleaching (Gotz et al. 2007; Mielczarek et al. 2008). These products are applied like medical plasters onto the surface of the labial tooth and lap over the incisal edges of the teeth. White et al. (2004) demonstrated that application of Crest Whitestrips gel containing up to 6.5% hydrogen peroxide for periods up to 70 hours produce no changes in the structure or architecture of enamel and dentin. Other over-the-counter systems include paint-on carbamide peroxide preparations with various concentrations used in a similar way to the bleaching strips. Disposable trays that are prefilled with 9% hydrogen peroxide with inbuilt gingival protection have been introduced as OTC to the market for direct use by patients. However, the main question is whether these systems really work especially as they are shown on television advertisements. Kugel & Kastali (2000) and Gerlach et al. (2002) compared the efficacy of using whitening strips containing 5.3% hydrogen peroxide versus placebo. They reported significant shade lightening during 2 weeks treatment with minor tooth sensitivity. Auschill et al. (2005) demonstrated acceptable bleaching results after bleaching with OTC, in office bleaching or at home bleaching. However, they cited that the most accepted method was the at-home bleaching technique in comparison to the other tested bleaching techniques (Auschill et al. 2005).

2.8 Bleaching materials

The main active agent in bleaching process is hydrogen peroxide. Products vary upon the concentration of the bleaching agent. It is either carbamide peroxide, which breaks down to form hydrogen peroxide, or hydrogen peroxide itself. Sodium perborate has also been used for internal bleaching that can release hydrogen peroxide through chemical reaction.
2.8.1 Hydrogen peroxide

Louis Jacque Thenard discovered hydrogen peroxide (H₂O₂) in 1818 (Aftalion 2001). Hydrogen peroxide is a chemical compound that contains the peroxide ion (O₂²⁻). The peroxide ion consists of a single bond between two oxygen atoms (O-O)²⁻. It is a strong oxidizer. Hydrogen peroxide (HP) has the structural formula: H-O-O-H (Fig.4).

![Fig.4: Hydrogen peroxide molecule with the structural formula (H-O-O-H)](Source: www.commons.wikimedia.org)

Compared to the more stable water molecule HP molecule contains one extra oxygen atom. The bond between the two oxygen atoms (the so-called peroxide bond) is broken while two H-O radicals are formed. These radicals quickly react with other substances, while new radicals are formed and a chain reaction takes place (Fig.5). HP is a liquid with a bitter taste and is highly soluble in water to give an acidic solution. HP is a relatively stable oxidant that is naturally present in the body (Spector 1990). At high concentrations these solution give off an irritating, acidic smell. It can disintegrate during transport releasing oxygen and heat. HP itself is inflammable, but the oxygen that results from its reaction can enhance the inflammation of other substances (Walsh 2000).

At low temperatures (-0.43 °C) HP becomes solid. The amount of HP in the solution is expressed in weight percentage.

Currently HP (CAS No.7722-84-1) is the primary material used for bleaching processes. HP is used for the production of chemicals and bleaching of cellulose pulp and textiles. Small concentrations are used for other purposes, e.g. as disinfection of eye contact lenses, disinfections of wounds and mouth washing.
Both HP and carbamide peroxide are used for hair bleaching, oral antiseptics, dentifrices, hair relaxer, ear drops and tooth bleaching. HP breaks down into oxidizing agents such as hydroxyl radicals and active oxygen (Farmer et al. 2006; Kashima-Tanaka et al. 2003). The figure (Fig.5) demonstrates the chemical reaction of HP. It is considered as antibacterial agent that has been gaining attention recently. It affects a wide range of organisms such as bacteria, yeast, fungi, viruses and spores (Block 1991). Bacteria that do not have catalase activity are particularly sensitive to HP because of its inability to break peroxide (Steinberg et al. 1999).

\[
\begin{align*}
(A) \quad & H_2O_2 \rightarrow 2HO^* \\
& HO^* + H_2O \rightarrow H_2O + HO_2^* \\
& HO_2^* \leftrightarrow H^* + O^*_2 \\
(B) \quad & 2H_2O_2 \leftrightarrow 2H_2O + 2{O} \leftrightarrow 2H_2O + O_2 \\
(c) \quad & H_2O_2 \leftrightarrow H^* + HOO^* \\
\end{align*}
\]

Fig.5: Hydrogen peroxide reactions. Step (A): Formation of free radicals like hydroxyl and perhydroxyl radicals and superoxide anions. (B): Formation of reactive oxygen molecules that are unstable and transform to oxygen. (C): Formation of hydrogen peroxide anions (Dahl & Pallesen 2003)

HP originates in the human body as a natural component in different metabolism processes. Nevertheless, it is considered as a cell poison due to the ability of radical production. These radicals can damage proteins and fatty
acids. Therefore, HP is deactivated quickly in the body tissues, e.g., in an enzymatic reaction by Peroxidase and Katalase (Boveris et al. 1972). On the other hand, macrophages and other defensive cells use HP in the phagocytose process against bacteria (Plekhova 2006).

### 2.8.2 Carbamide peroxide

Carbamide peroxide (Fig.6) (CAS No. 12-43-6), chemical formula (H$_2$NCONH$_2$-H$_2$O$_2$) separates into hydrogen peroxide and urea in contact with saliva or soft tissues at oral temperature (Kugel et al. 2007). Carbamide peroxide (CP) is an unstable solution.

\[
\text{H}_2\text{NCONH}_2 \cdot \text{H}_2\text{O}_2 \overset{\text{in water}}{\longrightarrow} \text{H}_2\text{NCONH}_2 + \text{H}_2\text{O}_2
\]

Fig.6: Formation of hydrogen peroxide from carbamide peroxide (Dahl & Pallesen 2003)

For example, 10% CP degrades into 3% hydrogen peroxide and 7% urea. The urea may provide some beneficial side effects because it tends to raise the pH value of the solution (Haywood & Heymann 1991). Urea is colorless, odorless, highly soluble in water and relatively non-toxic. It is neither acidic nor basic. Urea is produced naturally in the salivary glands and is present in saliva and the gingival crevicular fluid. Urea in bleaching products enhances anticariogenic effects, saliva stimulation and wound healing (Archambault 1990).

Carbamide peroxide is used as a disinfectant in the treatment of sores as well as in the nose-pharynx space. It is used in concentrations from 10-15% (Haywood & Heymann 1991). Gürgan et al. (1996) compared in vitro three bleaching products with 10% carbamide peroxide and 0.2% chlorhexidine as positive control in their effectiveness against caries bacteria (Streptococci and Lactobacilli). The bacterial
colonies were incubated for 24-48 hours with the substances. It appeared that the bleaching products were more effective in the inhibition of the growth of all species tested (S. mutans, S. sanguis, S. mitis, L. casei, L. acido-philus) compared to chlorhexidine (Gurgan et al. 1996). Bentlay et al. (2000) demonstrated in vitro that carbamide peroxide in the bleaching gels inhibited the growth of Streptococcus mutans after one hour and after two hours of Lactobacillus. Other study (Almas et al. 2003) found a statistically significant reduction of plaque and inflammation of the marginal parodontium during a three week therapy with 10% carbamide peroxide. Safety of CP as bleaching material has been reported in many studies (Berga Caballero et al. 2007; Moraes et al. 2006; Worschech et al. 2006)

### 2.8.3 Sodium perborate

Sodium perborate (CAS No. 7632-04-4) is unstable in water. Its main degradation and hydrolysis products are hydrogen peroxide and boric acid (Fig.7). Sodium perborate is used for internal bleaching after mixing with water.

\[
Na_2[B_2(O_2)_(OH)_4] + 2H_2O \rightarrow 2NaBO_3 + 2H_2O_2
\]

Fig.7: chemical reaction of sodium perborate (Dahl & Pallesen 2003)

### 2.8.4 Carbopol in bleaching materials

Several in situ gel forming systems such as polylactide-co-glycolide have been developed to prolong the precorneal residence time of a drug and improve ocular bioavailability (Dhawan et al. 2010). Polymers such as carbopol are involved in such delivery systems. Carbopol (carboxypolymethylene) is a polyacrylic acid polymer which can be used as a bioadhesive vehicle for drug delivery (Shojai 1998). Carbopol polymers are well known for their ability to
thicken, suspend and stabilize aqueous formulations. Carbopol is usually added to the bleaching products to control the release of oxidizing agents. The solutions containing Carbopol release oxygen slowly, while those without carbopol are fast oxygen-releasing solutions. Carbopol also enhances the viscosity of bleaching materials, which allows better retention of the slow releasing gel in the tray and on the tooth surface. Thicker products remain on the teeth to provide the necessary time for the carbamide peroxide to diffuse into the tooth (Greenwall 2001). The increased viscosity seems to prevent the saliva from breaking down the hydrogen peroxide, which might achieve more effective results (Greenwall 2001).

### 2.9 Safety aspects of tooth bleaching

#### 2.9.1 Soft tissues

Walking bleach technique is the most popular bleaching technique for non-vital teeth. The burning potential of soft tissues from relative high concentration of hydrogen peroxide (HP) is one of the main problems of walking bleaching technique (Walsh 2000). Therefore, a good sealing of the pulp chamber during bleaching treatment is necessary.

External bleaching techniques combined with extra activation by heat, light or laser may have an adverse effect on pulpal tissue (Buchalla & Attin 2007; Gokay et al. 2005, Slezak et al. 2002, Sulieman et al. 2005, Thitinanthapan et al. 1999). Most bleaching techniques advocate no use of heat or light for the reaction (Haywood 1991). Benetti et al. (2004) demonstrated that levels of HP in the pulp chamber depend on the concentrations of bleaching agent used in the treatment. The penetration of HP into the tooth pulp chamber from bleaching gels containing 10% CP (about 3.6 % HP) has been determined in vitro and found to be in the range of 0.339 to 3.605 mg after 25 min of treatment (Thitinanthapan et al. 1999).
However, the concentration of peroxide found in the chamber of tooth pulp after bleaching with 6% HP, which was 0.44 mM, is 3000 times below the concentration to cause pulpal enzyme damage (Joiner & Thakker 2004). Concerning the safety issue in home bleaching the carefully adapted plastic trays or nightguards may reduce the amount of whitening agent that is expelled onto the oral mucosa when the patient overfills the tray (Goldberg et al. 2010). On other hand, the use of a reservoir in the custom tray for home bleaching resulted in higher rates and higher intensity of gingival inflammation (Kirsten et al. 2009).

The safety of in-office bleaching of vital teeth using relative high concentration of HP has been widely investigated (Curtis et al. 1996, Fugaro et al. 2004, Robertson & Melfi 1980; Schulte et al. 1994; Seale et al. 1981). In vivo study on dogs (Seale et al. 1981) demonstrated histological adverse effects after bleaching with 35% HP for 30 min. However, the adverse changes were reversible after 60 day of the treatment.

There is a lack of clinical evidence regarding the safety and effectiveness of over the counter bleaching products (Demarco et al. 2009). Patients use over the counter bleaching products directly without control of dentist. Therefore, the unsupervised use of these products can lead to adverse effects, which can not be observed by dentist. The recent reviews (Goldberg et al. 2010; Matis et al. 2009) of the effectiveness of tooth whitening systems suggest that dentist-prescribed bleaching techniques was shown to be the most effective and safe method of bleaching.

2.9.2 Hard tissues

Bleaching teeth is considered a conservative treatment of tooth discoloration compared to other treatment possibilities concerning crowns and veneers. However, bleaching of non-vital teeth may induce resorption in the cervical area
in an unpredictable manner (Goldberg et al. 2010). Lado et al. found that heat and trauma during the bleaching process can cause a resorption (Lado 1988). In in-vivo study on dogs (Madison & Walton 1990) reported histological evidence of cervical root resorption and ankylosis after internal bleaching with 30% hydrogen peroxide (HP) associated with heat. However, the authors found no resorption with the internal bleaching group alone. These results may relate the heat associated with bleaching to the risk of root resorption after bleaching.

Concerning the safety of carbamide peroxide (CP) Ritter et al. (2002) reported the safety of 10% CP after 10 years post treatment with no noticeable side effects. Oltu & Gurgan (2000) found no change of enamel structure after bleaching with 10% and 16% CP. Another study (Turkun et al. 2002) found an alteration of the enamel structure immediately after bleaching with 10% carbamide peroxide. However, these changes were reversible 3 months after the treatment.

One of the most important clinical observations was the decrease in bond strengths of composite resin to bleached, etched enamel immediately after the bleaching process with 35% HP (Titley et al. 1988). The presence of residual peroxide or peroxide-related substances at or near the enamel surface may be the cause for the decrease of adhesion strength (Titley et al. 1991; Torneck et al. 1990).

2.9.3 Dental sensitivity

Tooth sensitivity is a common side effect of tooth bleaching with carbamide peroxide (CP) and hydrogen peroxide (HP) (Haywood 2005; Swift 2006), which is usually reversible after the end of treatment (Alomari & El Daraa 2010). To reduce the patient complaints, some researchers (Haywood et al. 2001; Haywood et al. 2005) suggested one or more of the following procedures for the treatment and prevention of sensitivity:

- Inclusion of desensitizing agents in the bleaching materials (for example potassium nitrate)
• Use of desensitizing dentifrices
• Placement of fluoride solutions in the custom trays for topical application
• Decrease the exposure to bleaching gels (for example, bleaching for one- to two-hour periods rather than overnight)
• Use of ingested analgesics

The general clinical cause of tooth hypersensitivity is exposed dentinal tubules as a result of gingival recession and subsequent loss of cementum on root surfaces. However, during teeth bleaching, the theory of sensitivity is that the oxygen molecules released from CP or HP diffuse through and accumulate in the enamel and dentin. When a sufficient amount of the substance occupies the intracoronal space, pressure could be applied to pain receptors in the dentinal tubules and to the pulp. Higher concentrations of bleaching solutions cause more sensitivity than those with lower concentrations (Croll 2003; Jacobsen & Bruce 2001). The most affected patients range in the age from 20 to 40. The facial surface of cupids, premolars and incisors tend to be most sensitive teeth (Stojsin et al. 2008).
3 Plan of the study

- Bovine teeth
- Preparation of enamel samples Ø 5 mm
- Finishing the samples
- Bleaching groups
- Control group (n=10)
- Roughness measurement (Ra, Rt, Ry, RzDIN) (baseline)
- Microhardness (baseline)
- Bleaching groups
- Carbamide peroxide (CP) A =10% n =10
- Hydrogen peroxide (HP) B=35% n =10
- C=3.5% n =10
- D=10% n =10
- - Bleaching for two hours every second day
- - Fresh material every 40 min
- - Frequency of application 6 times within two weeks
- Storing samples during the experiment in artificial saliva at room temperature
- Roughness measurement (Ra, Rt, Ry, RzDIN)
- Microhardness
- Statistical analysis
4 Materials and methods

4.1 Preparation of the enamel samples

Bovine central upper incisors were stored in 0.1% thymole solution at room temperature (Kugel et al. 2007). The teeth were cleaned from debris and stored in dematerialized water for 24 hours. Teeth that had any visible crack or hypoplastic defect were excluded. Only crowns of the teeth were used in this study. The roots were sectioned at the cemento-enamel junction using a slow speed rotary saw (Exakt GmbH, Norderstedt, Germany) with water cooling. The pulp was removed from its chamber using a dental probe. Bovine enamel samples prepared from the buccal surfaces of the teeth were embedded in cylindrical acrylic resin (Technovit, Heraeus Kulzer, Wehrheim, Germany) forms (Ø 2.5 cm) and finished with sand papers 1200, 2400, 4000 (Sic-paper, Struers, Denmark) and polishing paste 0.1µm (AP-D Suspension, Struers, Denmark). All samples were finished using a polishing vacuum-device (Exakt, Norderstedt, Germany) to control the parallelism of the sample surface. The enamel surfaces were examined with a light microscope (50x, Axioskop 2 MAT, Carl Zeiss, Goettingen, Germany) for any defect on the surface after finishing.
Fig. 8: Enamel surface (50 x magnifications) appears smooth with some scattered clear scrapes (black arrows) due to polishing procedure.

4.2 Preparation of the artificial saliva

The artificial saliva was made as described previously (Klimek et al. 1982). The composition of the saliva is shown in (Table 1)

The solution was stirred till dissolution of the all substances. Each experimental tube was filled with 20 ml artificial saliva for storing the samples (one sample in every tube).
Table 1: Chemical composition of the artificial saliva used as storing solution

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.030 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.580 g</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.170 g</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>0.160 g</td>
</tr>
<tr>
<td>KCl</td>
<td>1.270 g</td>
</tr>
<tr>
<td>NaSCN</td>
<td>0.160 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.330 g</td>
</tr>
<tr>
<td>urea</td>
<td>0.200 g</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>0.340 g</td>
</tr>
<tr>
<td>Aqua desta</td>
<td>1000ml</td>
</tr>
</tbody>
</table>

4.3 Microroughness measurement

4.3.1 Laser profilometer

Fig. 9 shows the UBM device (Mikrofocus, UBM, Type 2010, UBM, Karlsruhe, Germany) with autofocus sensor and x/y-measurement table. The tested sample is positioned on the x/y table and then measured using the laser sensor. The measurement protocol (Table.2) and the control of sensor position were controlled by the provided software (UBSOFT, version 1.909).
4.3.2 Roughness parameters

Roughness changes of the enamel surface were measured by the dynamically focusing optical UBM profilometer before and after the bleaching. To measure the initial surface roughness before bleaching (baseline), three different areas (0.7x0.7 mm) on every sample were used to perform three measurements with 1000 points/mm as pixel density of the scanned surface. Dimensions of the measured area were controlled by UB_SOFT software, beginning from the center of tested surface. A laser beam was used as an optical stylus. The sample was moved with stepper motors provided by the profilometer. Three microroughness parameters were used for description of recorded profiles: Ra, Rt-Ry, Rz (see capital roughness parameters)
Materials and methods

<table>
<thead>
<tr>
<th>Measurement range</th>
<th>± 50μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimension of measured surface</td>
<td>0.7 x 0.7 mm</td>
</tr>
<tr>
<td>Point density of measurement (x)</td>
<td>1000 P/mm</td>
</tr>
<tr>
<td>Point density of measurement (y)</td>
<td>1000 P/mm</td>
</tr>
<tr>
<td>Speed of measurement</td>
<td>0.5 mm/S</td>
</tr>
<tr>
<td>Frequency of measurement</td>
<td>500 P/S</td>
</tr>
</tbody>
</table>

Table.2: Measurement protocol of the UBM software used in this study to investigate the roughness parameters of enamel samples

- Measurement range: the range of the scanned surface topography in Z dimension.
- Dimension of measured surface: three areas on every enamel surface were measured. The dimension of every scanned area on the sample is shown in this field.
- Speed of measurement: the speed of sensor along the scanned area.
- Frequency of measurement: the number of measured points in seconds.

4.4 Surface micromorphology

4.4.1 Scanning electron microscope (SEM)

In comparison to the light microscope SEM has a large depth of field, which allows a relative large range of the sample to be in focus at one time. A large depth of field is sometimes a desirable property of a microscope, for example, when observing rough surfaces (Sheppard & Wilson 1978). SEM also produces images of high resolution. Closely spaced features can be examined at a high magnification. The samples are prepared to be conductive with vacuum device (Bal-Tec GmbH, Schalkmühle, Germany) as follow:
- Sputter of gold water (19.3 g/cm$^3$) at a working pressure of 0.05 mbar, working distance of 50 mm, sputter current 60 mA, Sputter time of 40 sec. According to this setup, a gold layer of 20 nm was obtained on the surface (Fig.12). The samples were examined with SEM (LEO 435 VP, LEO Elektronenmikroskopie GmbH, Oberkochen, Germany)

![Image](image.png)

Fig.10: Preparation of SEM samples with 20 nm layer of gold to be conductive

### 4.5 Microhardness measurement

Knoop microhardness tester (Leitz Miniload, Ernst Leitz GmbH, Wetzlar, Germany) (Fig.11) was used to test the surface microhardness. The surface of the sample was placed parallel to the plate of microhardness device (Fig.12). A load of 1030 mN was used with a loading time of 30 seconds. Two microhardness measurements from two random positions on the upper surface of each sample were performed before (baseline) and after bleaching procedures.
Materials and methods

Fig. 11: Knoop microhardness tester

Fig. 12: Measurement the enamel microhardness of the samples
4.6 Bleaching procedures

Samples were divided in five groups (A-E). Group A was treated with 10% CP, group B with 35% CP, group C with 3.6% HP and group D with 10% HP (Batch Nr. BZ 008C). Group E was as negative control group (no bleaching).

The enamel specimens of each group were bleached, with the corresponding bleaching material, with the same protocol: two hours every second day, during a two weeks period. Bleaching gel (0.1ml) was applied with a graduated syringe, stirred every 10 minutes ensuring continuous contact of fresh material to the tested surface. The procedure was repeated three times within two hours. Samples were washed with water for 10 seconds followed by drying with pressed air for 5 seconds before application of fresh gel. During the experiment, all samples were stored in artificial saliva.

4.6.1 Bleaching materials

Four experimental bleaching materials were delivered from the same manufacture (Ultradent products, Inc. S. South Jordan, UT USA) with following concentrations:

- 10% CP (Batch Nr. BZ 008A)
- 35% CP (Batch Nr. BZ 008B)
- 3.6% HP (Batch Nr. BZ 008D)
- 10% HP (Batch Nr. BZ 008C)

Fig.13: All tested materials were delivered in form of gel. It was applied through graduated syringe to control the amount of bleaching gel used on the samples.
All materials were delivered according to the plan of the study in form of colorless gels (Fig.13). The gels differed concerning their content of CP or HP, respectively. All gels contained the same components: approximately 20% water, CP or HP, less than 3% of carbomer, sodium hydroxide, stabilizers and buffers.

All tested products were stored in +4°C. The tested gels were controlled to have room temperature before application. PH values were measured with digital pH meter (inoLab pH 720, WTW, Weilheim, Germany) after calibrating with two standard buffers pH=7 and pH=4.01 at 25°C (Technical Buffer, WTW, Weilheim, Germany). PH value was measured by covering the electrode with the tested gel. The electrode was cleaned from bleaching gel with demineralized water. The four experimental bleaching gels had a pH between 6.47 and 6.96 at room temperature. According to the instruction of the manufacturer all gels were delivered ready to use with no need for activation.

4.7 Statistical analysis

The data of measured roughness parameters showed a normal distribution. All tested parameters were significantly different (p=0.0001) before the bleaching. Therefore, roughness parameters ($\Delta$Ra, $\Delta$Rz, $\Delta$Rt-Ry) after bleaching, compared to baseline before bleaching, were calculated.

Roughness and microhardness results were statistically analyzed. An analysis of covariance (ANCOVA) was used. The response variable was the change that was calculated by subtracting the values after bleaching from the baseline values before bleaching ($\Delta$ value). The group effect was calculated with controlling for the baseline values as covariate. Least-square means with 95% CI were calculated for each group. Adjustment for Multiple Comparisons was done by the method of 'Dunnett-Hsu'.
5 Results

5.1 Microroughness

The results of roughness of enamel surface were summarized in the Table 3 after bleaching with the tested material in this study.

<table>
<thead>
<tr>
<th>Bleaching gels</th>
<th>ΔRa (µm)</th>
<th>ΔRz(µm)</th>
<th>ΔRt-Ry(µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35% CP</td>
<td>0.014</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>10% CP</td>
<td>-0.003</td>
<td>-0.05</td>
<td>-0.07</td>
</tr>
<tr>
<td>10% HP</td>
<td>0.017</td>
<td>0.17</td>
<td>0.18</td>
</tr>
<tr>
<td>3.6% HP</td>
<td>0.002</td>
<td>0.02</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table 3: Δ values demonstrate the increase (positive values) or decrease (negative values) of the three tested roughness parameters (Ra, Rz, Rt-R-y) after bleaching with carbamide peroxide (CP) or hydrogen peroxide (HP). P-values of the significant changes are mentioned.

Roughness average Ra

Statistical analysis (Fig.14) demonstrated that bleaching procedures in this study had a significant effect on ΔRa (p=0.0001). The group D (10% HP) compared to the group E (control group with no bleaching) showed significantly higher ΔRa (p=0.01). The group B (35% CP) showed only the tendency of higher ΔRa value (p=0.056) compared to the baseline before bleaching. No significant changes of ΔRa were observed after bleaching concerning groups A (10% CP) and C (3.6% HP). Comparison of group A (10% CP) vs. group C (3.6% HP) and group B (35% CP) vs. group D (10% HP) demonstrated no statistical changes in ΔRa value.
Roughness average in Z dimension – RzDIN

Bleaching procedures in this study had a significant effect on ΔRz (p=0.0001). The 10% HP group showed significantly higher ΔRz compared to the control (p=0.04). No significant difference in ΔRz values were observed after bleaching with 35% CP or 10% HP or 3.5% HP compared to the baseline before bleaching (Fig.15). However, bleaching with 10% CP showed significantly lower ΔRz.
with -50nm (p=0.03). Comparison of 3.5% HP vs. 10% CP and 10% HP vs. 35% CP demonstrated no statistical change in $\Delta R_z$ value.

Fig.15: Roughness average ($\Delta R_z$) in $\mu$m, after bleaching with 10% carbamide peroxide (CP), 35% CP, 3.6% hydrogen peroxide (HP), and 10% HP. $\Delta R_z$ is significantly decreased after bleaching with 10% CP (p=0.02). Bleaching with 10% HP increased $\Delta R_z$ (p=0.04). No significant difference is observed after bleaching with 35% CP or 3.6% HP. P-values are given for significant changes (before-after bleaching).

Roughness depth $\Delta R_t$-$R_y$

Bleaching procedures had a significant effect on $\Delta R_t$-$R_y$ (p=0.002). Bleaching of enamel surface with 10% CP showed significantly lower $\Delta R_t$-$R_y$ with -70 nm (p=0.05) compared to the baseline before bleaching. No significant change was
Results

observed after application of 3.6% HP, 35% CP and 10% HP (Fig.16). $\Delta R_t-R_y$ showed significant differences between 3.5%HP vs. 10% CP ($P=0.03$). $\Delta R_t-R_y$ was lower in the 10% CP group ($p=0.05$).

Fig.16: Maximal roughness depth ($\Delta R_t-R_y$) in µm, after bleaching with 10% carbamide peroxide (CP), 35% CP, 3.6% hydrogen peroxide (HP), and 10% HP. $\Delta R_t-R_y$ is significantly decreased after bleaching with 10% CP ($p=0.05$). No significant difference is observed after bleaching with 35% CP, 10% HP or 3.6% HP. P-values are given for significant changes (before-after bleaching).

5.2 Micromorphology

Laser profilometer was used to show the 3D changes of the enamel surface in this study. Scanning electron microscope (SEM) provides a 2D view of the enamel surface. The enamel surface of control samples appeared smooth in
Results

general with some scattered clear scrapes due to the polishing procedure (Fig.14 A,B)

Fig.17: (A) 3D view of enamel surface morphology (0.7x0.7mm) before bleaching scanned with laser profilometer (1000 point/mm). (B) 2D view of enamel surface before bleaching, scanned with SEM (magnification x 3000).
5.2.1 Bleaching with 10% carbamide peroxide

The enamel surface after treatment with 10% CP showed only minimal surface changes compared to the control (Fig.18 A,B).

Fig.18: (A) 3D view of enamel surface morphology (0.7x0.7mm) after bleaching with 10% carbamide peroxide scanned with laser profilometer (1000 point/mm). (B) 2D view of enamel surface scanned after bleaching with 10% carbamide peroxide with SEM (magnification 3000 x)
5.2.2 Bleaching with 35% carbamide peroxide

Although there was statistically just slightly higher ΔRa after bleaching with 35% CP, surface changes could be distinguished in surface profile, especially with the SEM figures, in comparison to the control (Fig.19 A,B).

Fig.19: (A) 3D view of enamel surface morphology (0.7x0.7 mm) after bleaching with 35% carbamide peroxide scanned with laser profilometer (1000 point/mm). (B) 2D view of enamel surface scanned after bleaching with 35% carbamide peroxide with SEM (magnification 3000 x)
5.2.3 Bleaching with 3.6% hydrogen peroxide

The enamel surface after treatment with 3.6% HP showed minimal surface changes compared to the control (Fig.20_A,B). However, some of the randomly depression spots were remarkable in SEM photos after application of 3.6% HP (Fig.20_B) in comparison to 10% CP Fig.18_B).

Fig.20: (A) 3D view of enamel surface morphology (0.7x0.7mm) after bleaching with 3.6% hydrogen peroxide scanned with laser profilometer (1000 point/mm). (B) 2D view of enamel surface scanned after bleaching with 3.6% hydrogen peroxide with SEM (magnification x 3000)
5.2.4 Bleaching with 10% hydrogen peroxide

Bleaching with 10% HP resulted in qualitative changes of the surface morphology compared to the control specimens (Fig.21_A,B). Enamel appeared to have intermittent depressions of various diameters and depths. Random scratches were also detected. According to the SEM figures, the severity of changes due to 10% HP (Fig.21_B) seemed to be more pronounced than to 35% CP (Fig.19_B).
Fig. 21: (A) 3D view of enamel surface morphology (0.7x0.7mm) after bleaching with 10% hydrogen peroxide scanned with laser profilometer (1000 point/mm). (B) 2D view of enamel surface scanned after bleaching with 10% hydrogen peroxide with SEM (magnification x 3000)
5.3 Microhardness

Enamel microhardness (ΔMic) was significantly higher after bleaching with 10% HP (ΔMic=+20 KHN, p=0.0002) and 35% CP (ΔMic=+8 KHN, p=0.01) compared to the baseline. No significant change of enamel microhardness was observed after application of 10% CP or 3.6% HP.

Fig.22: Microhardness results (ΔMic) in KHN, before, after bleaching with 10% carbamide peroxide (CP), 35% CP, 3.6% hydrogen peroxide (HP), and 10% HP

<table>
<thead>
<tr>
<th>Bleaching gels</th>
<th>35% CP</th>
<th>10% CP</th>
<th>10% HP</th>
<th>3.6% HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔMic (KHN)</td>
<td>8.4 (p=0.01)</td>
<td>-3.4</td>
<td>20 (p=0.0002)</td>
<td>-7.5</td>
</tr>
</tbody>
</table>

Table.4: Δ Mic demonstrate the increase (positive values) or decrease (negative values) of the enamel microhardness (Mic) after bleaching with carbamide peroxide (CP) or hydrogen peroxide (HP). P-values of the significant changes are mentioned.
In the past decade, numerous studies have evaluated the effects of bleaching agents on teeth. However, there is no agreement about the effect of bleaching materials on the physical properties of dental tissues. This may be due to the difference of the concentrations of bleaching agents used (carbamide peroxide or hydrogen peroxide) and the applied technique. The aim of this in vitro study was to assess the micro changes of enamel surface in three dimensions using laser profilometer. Scanning electron microscope photos were performed for additional comparison. Enamel microhardness changes were also estimated using knoop microhardness tester.

6.1 Discussion of the materials

6.1.1 Preparation of samples

Bovine teeth were used in this study. They have been used in previous in-vitro studies. According to Nakamichi et al. (1983) and Reeves et al. (1995) bovine teeth are similar to human teeth in their composition and can be used as a substitute of human teeth.

6.1.2 Storage solution

The most common storing solutions for in-vitro studies are saliva and demineralized water.

In this study, we used the artificial saliva because it is considered to cause no change in surface roughness of enamel (Abouassi et al. 2008). The content of artificial saliva is introduced in relevant to natural saliva concerning the mineral and organic content. Therefore, pH and the mineral content of saliva
can protect the enamel surface from potential negative effect through storage time in vitro. This theory is confirmed from the author in pre-experimental trial (Abouassi et al. 2008). The investigation compared the used artificial saliva in this study with demineralised water. Saliva was the optimal choice concerning the aim of this study.

6.1.3 Bleaching materials

Bleaching materials come into direct contact with the tooth surface for a rather long period of time depending on the technique. Therefore, it is important to know the effect of bleaching on tooth structure. With respect to the bleaching agents, many factors were standardized (commercial components of tested products, time of bleaching and pH of these materials) to make sure that any changes on enamel surface will be due of hydrogen peroxide (HP) or carbamide peroxide (CP). HP is used in dentistry as a whitening material at different concentrations from 5%–35% (Plotino et al. 2008). The chemical reaction of 10% and 35% CP release about 3.6 and 10%HP, respectively. CP and HP were used in the present study in the corresponding concentrations to compare their effect on the enamel surface.

Sulieman et al. (2004) reported the adverse effects of bleaching materials on enamel and dentine because of pH changes of the formulation used, but not the bleaching agent itself. In another study 10% CP was compared with different pH values. It was demonstrated that the most severe alterations were found in slabs exposed to the lower pH solutions after 4 weeks (Shannon et al. 1993). To avoid this effect, all tested materials were above the critical pH value (>5.5) for tooth mineral dissolution (Cairns et al. 2002). The measured pH values for all materials were between 6.47 and 6.96. The only difference between these materials was either the kind of bleaching agents (CP or HP) or their concentration. High concentration of carbamide peroxide (35%) is indicated as
pre-treatment and in association with at-home vital bleaching. These products are for professional use only.

Bleaching materials tested in this study were applied on the enamel surface without any activation. The materials were provided ready to use. Many studies reported an additional trauma of teeth tissue and an increase of risk of external cervical resorption by using external activation such as heat or light (Attin et al. 2003; Buchalla & Attin 2007; Dias Ribeiro et al. 2009). Therefore, any external activation of tested material was not performed in this study. Previous studies signed bleaching with no extra activation as sufficient treatment (Matis et al. 2009; Valera et al. 2009).

6.2 Discussion of the methods

6.2.1 Time of bleaching

Time needed for bleaching procedures depends on the technique and concentration of the materials. For the application of home bleaching techniques about 10% CP is utilized four up to six hours per day in a period of six weeks (dos Santos Medeiros & de Lima 2008), whereas 35% CP is suggested as initial treatment applied in office to accelerate the bleaching results (Efeoglu et al. 2007). In this study, all tested experimental groups were treated using the same treatment protocol (2 hours every 2 days during two weeks) to avoid any effect of bleaching time on the surface roughness of enamel between the groups.

6.2.2 Protocol of bleaching

Some studies demonstrated that 10% and 35% carbamide peroxide did not alter the enamel surface roughness, but tooth brushing during bleaching could
significantly increase the enamel surface roughness (Worschech et al. 2006; Worschech et al. 2003). Therefore, no other erosive or abrasive procedure such as tooth brushing was applied on the enamel surface during the study.

### 6.2.3 Laser profilometer

Object topography in nature is three-dimensional, so measurement and analysis of two-dimensional profile may be incomplete to real topographical changes of tested surfaces (Kocher et al. 2002). Laser profilometer was involved in the study to investigate microroughness and micromorphology quantitatively and qualitatively. Non-contact optical profilometer was described as an effective method for 3D analysis of the surface morphology of the tooth surface (Kocher et al. 2002). Scanning of the surface was performed using a sensor with laser light. So, no mechanical defect could be generated on the measured surface. The advantage of the auto focus procedure is its high exactness and the auto controlling of the measurement speed which is important for roughness estimation. In this study, laser profilometer is considered as an accurate method to investigate surface roughness. Kocher et al. reported that Ra is highly dependent on the area of the field to be scanned, increasing linear with the measured area (Kocher et al. 2002). Therefore, all measured areas were achieved according to the same protocol with respect to the field size and pixel density in the current study.

### 6.2.4 Roughness parameters

The roughness parameter Ra describes the overall roughness of a surface and is defined as the arithmetic average value of all absolute distances of the roughness profile from the centerline within the measured length. Although the Ra is the most important parameter to evaluate roughness, Whitehead et al. (1995) suggested that the surface characteristics should be identified using
more than one surface measurement parameter. The authors demonstrated that other parameters may be used as complementary data to obtain more information about the profile shape of the surface. Ra is the most frequently reported parameter to measure surface roughness within dental studies. The additional roughness parameters allow further description of surface quality (Field et al. 2010). Therefore, in addition to Ra, Rz and Rt-Ry were estimated in the present study.

The sampling matrix, which is the number of pixels in the x and y dimensions, also has an effect on the measurement value. Therefore, a few numbers of pixels will not contain enough information from the surface to provide reliable surface parameters. In the current study, all measured areas were achieved with the same protocol with respect to the field size and pixel density. Pixel density for scanning the surface roughness in our study was set to 1000 pixel/mm based on the Kocher et al. study (Kocher et al. 2002). They found that Ra value approaches had a maximum beyond 400pixel/mm. Because there are little changes in the surface roughness on the enamel surface after bleaching increasing the pixel density could provide more accurate information of the roughness of surface. The outer surface of the enamel does not have clear morphology (Hellwig et al. 2009). So plan, parallel and well-polished sample surfaces are required to compare roughness measurement. For minimal interface of defect areas with roughness parameters, all samples were examined after preparation with light microscope (50x) to analyze the surface of any defect or cracks.
6.3 Discussion of results

6.3.1 Enamel roughness

In this study, the results of laser profilometry demonstrated that ΔRa and ΔRz were significantly higher after application of 10% HP compared to the control, while 35% CP showed only a slightly increase in roughness (ΔRa, p=0.056). The SEM pictures supported these results with a qualitative increase of roughness. In contrast to our results, Sulieman et al. (2004) reported no changes on the enamel surface after applying 35% HP using a profilometer. They used coarse paper for finishing the surface (800 grit), and the mean surface roughness was within ±0.3 µm at baseline. In our study, enamel surfaces were polished with ultra-fine wet grinding paper 4000 and 0.1µm polishing diamond paste, followed by optical observation under light microscope (50x). The present baseline surface roughness was within ±0.03 µm. Therefore, it was possible to observe the minimal changes in this study (i.e. after bleaching with 10% HP, the ΔRa was 0.017 µm, see the results section).

An X-ray-diffraction study by Kawamoto and Tsujimoto (2004) revealed that the structure of hydroxyapatite (inorganic part) did not change by 30% H₂O₂ treatment for one week at room temperature. These results showed that H₂O₂ did not damage the inorganic tissue. Another study (Hegedus et al. 1999) supported this theory, and showed that hydrogen peroxide attack the organic component of tooth structures. Hydrogen peroxide can penetrate into the enamel due of its low molecular weight. Thus, inner oxidative effects are more likely to occur in the subsurface enamel where more organic material is present. The dissolution of the organic part of low mineralized spots on the surface and between enamel prisms result probably in the morphologic changes registered by laser profilimeter in the present study. Jiang et al. (2008) stated also that HP is able to penetrate enamel structure and suggested that the organic material in enamel could be affected by the oxidation reaction of HP.
Application of lower concentrations of bleaching agents (10% CP or 3.6% HP) revealed no significant different values of the roughness parameters. Oltu & Gurgan (2000) revealed that tooth bleaching with 10% and 16% CP for 8 h per day for 6 weeks do not lead to any change of the enamel structure. In the same study, in which infrared absorption spectroscopy and X-ray diffraction analysis were used, application of 35% CP 30 min a day for a 4-day period was safe with no changes in the enamel structures. Another study (Turkun et al. 2002) investigated in vivo the effect of teeth bleaching on the enamel morphology by obtaining epoxy resin replicas from the maxillary right central incisors of the subjects before, immediately after, and 3 months after the bleaching procedures. They demonstrated an increase in the enamel porosity immediately after bleaching with 10% CP. However, these changes were reversible after 3 months of the treatment. These reversible changes could be explained by the suggestion that the changes in the surface morphology caused by CP may undergo repair over time by precipitation of mineral phases derived from saliva into the surface porosities (Flaitz & Hicks 1996). In our study, enamel surface was smoother after bleaching with 10% CP with significant changes of $\Delta R_{t-R_y}$ and $\Delta R_z$, but no change was observed concerning $\Delta R_a$ compared to the baseline. This may be due to the morphologic changes after bleaching. However, using SEM, spots with minimal morphological changes could be observed on the enamel surface after bleaching also with low concentrations (Fig.18_B, Fig.20_B).

Moreas et al. (2006) also registered a significant increase of enamel surface roughness after bleaching with 35% CP, but no change after bleaching with 10% CP. The authors investigated one parameter of surface roughness ($R_a$), whereas in our study significant smoother surfaces after bleaching with 10% CP could be revealed through roughness parameter $R_{t-R_y}$ and $R_z$. Field et al. (2010) demonstrated that $R_a$ is the main reported measurement as roughness parameter within dental studies and the additionally roughness parameters
will allow a further description of the surface quality. Our study agreed the results of Field et al. that measurement of more roughness parameters could deliver more information about the investigated enamel surface.

In another in vitro study (Markovic et al. 2007) confocal laser scanning microscopy was involved in the measurement of the microroughness of enamel surface after bleaching. The authors measured Ra and Rt roughness parameters before (the baseline was Ra=0.2 µm, Rt= 3.17 µm) and after bleaching. They demonstrated significant increase of the Ra and Rt after bleaching with 10% CP (Δ Ra= 0.09 µm) or 16 %CP (no exact data for Δ Ra). However, in focus on the material and methods, the measured enamel surface was used directly without finishing. The measured areas were 140 × 100 µm on three different areas on each sample. Therefore, the difference in material and methods of studies could result in different values of these roughness parameters. As mentioned previously, any change of the dimensions or scanning density of measured surface could result in changes of value of roughness parameters.

Decomposition of CP produces urea that theoretically decomposes to carbon dioxide and ammonia. Urea is capable of penetrating into enamel, affecting the interprismatic regions (Cavalli et al. 2004). Thus, urea might raise the pH value of bleaching materials and reduce any adverse effects due to its alkaline property (Cavalli et al. 2004). This reaction will help protecting against the acidic environment of HP reaction. These results suggest that using 10% HP through chemical reaction from CP might be safer than direct application of 10% HP on enamel. The present measurements resulted in little less roughness changes of the enamel surface after bleaching with CP compared to bleaching with HP concerning Rz values, and concerning the qualitative analysis with SEM. Jiang et al. (2008) supported the ability of HP to penetrate enamel structure and suggested that the organic matter in enamel might be greatly
affected by HP reaction. Therefore, the dissolution of the organic part of low mineralized spots on the surface and between enamel prisms has caused the morphologic changes registered with SEM for all bleaching concentrations in this study. Also, it has been suggested that the chemical reaction during the bleaching process does not attack the inorganic tissues (Hegedus et al. 1999). This assumption is supported by the fact that microhardness did not decrease in the present study.

Patel et al. (2008) reported that the efficacy of bleaching teeth with relative low concentrations of bleaching materials (10% CP) is comparable to the bleaching results obtained from high concentrations (35% CP). Therefore, and according to the results in our current study, bleaching teeth with low concentration of whitening materials (HP or CP) could be safer concerning the adverse effect of bleaching on enamel structure.

6.3.2 Enamel microhardness

The dissolution of the organic part of low mineralized spots on the surface and between enamel prisms have caused the morphologic changes observed using SEM for all bleaching concentrations in this study. The present study supports this theory as tooth bleaching had no negative effect on the enamel microhardness. As hardness is generally associated with salivary remineralization process, remineralization potential exists in saliva substitutes that contain calcium and phosphate (Shannon et al. 1993) as in this study. Artificial saliva has been used as storing solution and was suggested to repair any demineralization during bleaching procedures (Rodrigues et al. 2003). In comparison to the control group, a significant higher value of enamel microhardness was observed after bleaching with 10% HP and 35% CP. This might be caused according to the conditioning effect of weak acids (bleaching materials) which might increase the surface energy. This results in better
remineralization reaction during storage in artificial saliva. Additionally, the theory that the peroxide-containing bleaching agents affect the organic phase of enamel (Hegedus et al. 1999) may also increase the enamel microhardness. The destruction of this weak organic phase between the enamel prisms may be repaired through the remineralization effect of storing solution used in this study.

According to the Mahringer et al. (2009) the main change due of bleaching treatment occur in the first hour of bleaching and no significant change was observed after the next 6 hours of bleaching. We suggest that the great bleaching effect on enamel surface was in the first hours of bleaching, mainly on the organic part of enamel. The storage time in artificial saliva (till the end of study) was enough to repair the supposed decrease in enamel surface. As the samples were stored in artificial saliva after every bleaching treatment cycle, calcium-phosphate precipitation occurs inside the porous enamel, a phenomenon that leads eventually to rehardening enamel surface. Borges et al. (2009) investigated the potentially protect effect of calcium and fluoride within 35% HP bleaching gel. They found that the bleaching group with calcium-added agent exhibited increase in the enamel microhardness directly after 30 min. duration of bleaching. In contrast to our study the storing solution was the source of calcium as a remineralizing agent.

It has to be emphasized that this study was performed in vitro and that in the oral cavity additional important factors exist. Antioxidant enzymes and organic parts of the neutral saliva may affect the present observations.

Attin et al. (2007) showed in an invitro study that post-bleaching re-hardening of enamel surfaces is supported by the use of fluoridated 10% CP. However, they demonstrated also microhardness recovery of enamel surface after bleaching with no fluoride CP with neutral pH after 10 days. According to their
study protocol, they submitted all samples to the re-deminerlization cycle during and after bleaching enamel samples. In our study no additional erosion effect through or after bleaching procedure was applied. This may in turn explain the increased microhardness that observed in this study.

How to store bleached specimen in vitro studies seems to be an important factor for variations in the results concerning the microhardness of enamel surface (Abouassi et al. 2008). When neither saliva nor a buffered liquid was used to store specimens during bleaching protocols, a reduction in microhardness was observed (Park et al. 2004). However, when the specimens were stored in artificial saliva or in the oral medium in situ, no alteration of the surface hardness of enamel was registered (Attin et al. 2009; Faraoni-Romano et al. 2008; Maia et al. 2008).

Rodrigues et al. (2005) demonstrated in situ study a small reduction of microhardness directly after bleaching. According to their study, these changes seem to be clinically insignificant due to the small reduction. On the other hand, cabopol resulted in a similar reduction of enamel microhardness. These findings may introduce CP and HP as safe materials concerning the mineral part of teeth hard tissues.
7 Conclusions

Summarizing the results about the influence of bleaching agents (carbamide peroxide vs. hydrogen peroxide; low versus high concentrations of the bleaching agents) on the micromorphology, microroughness and microhardness of the enamel surface, it can be concluded that:

- The morphological alterations of the enamel surface observed after bleaching were irregular and varied with the concentrations used.
- Minimal increase of microroughness was registered after bleaching with relatively high concentrations of bleaching agents.
- Enamel surfaces that were treated with carbamide peroxide showed minimally less alterations compared to hydrogen peroxide.
- There was no decrease concerning enamel microhardness in any of the bleaching groups.

The main effect of bleaching agents on the enamel surface is thought to relate to the oxidation and subsequent partial lysis of the organic material and not to the dissolution of inorganic material.
8 Summary

The aim of the study was to investigate changes in the micromorphology and microhardness of the enamel surface after bleaching with two different concentrations of hydrogen peroxide (HP) and carbamide peroxide (CP). Bovine enamel samples were embedded in resin blocks, and polished. Specimens in the experimental groups (n=10) were treated with bleaching gels containing 10% CP, 35% CP, 3.6% HP and 10% HP, respectively, for two hours every second day over a period of two weeks. The gels had the identical composition and pH and differed only in their HP or CP content. The roughness and morphology of the enamel surface were analyzed using laser profilometry and SEM. Microhardness was measured using a Knoop-hardness tester. The data were evaluated statistically.

Specimens in the 10% HP group showed significantly higher roughness after bleaching compared to the control group (ΔRa, p=0.01). Bleaching with 35% CP showed only a tendency to increase roughness (ΔRa, p=0.06). Application of 10% CP or 3.6% HP had no significant influence on the roughness of enamel. Enamel microhardness was significantly higher after application of 10% HP (ΔMic=8 KHN, p=0.002) and the 35% CP (ΔMic=20 KHN, p=0.01) compared to the control group.

In summary, application of CP and HP showed only small quantitative and qualitative differences concerning surface alterations. Only bleaching teeth with relatively high concentrations of bleaching agents showed an increase in enamel roughness. All agents tested had no negative effect on microhardness of enamel. The observed effects of bleaching agents on the enamel surfaces are probably clinically irrelevant. Therefore, these findings support the statement about bleaching teeth as a safe, minimally invasive treatment.
Ziel der Studie war die Untersuchung der Mikrohärte und der Mikromorphologie der Schmelzoberfläche nach dem Bleichen mit zwei verschiedenen Konzentrationen von Wasserstoffperoxid (HP) im Vergleich zu Carbamidperoxid (CP).


Die 10% HP Gruppe zeigte verglichen mit der Kontrollgruppe (ohne Bleichen) eine signifikant höhere Rauhigkeit (ΔRa, p=0,01). Bei der 35% CP Gruppe zeigte sich nur eine tendenzielle Erhöhung der Rauhigkeit durch das Bleichen (ΔRa, p = 0,06). Die Anwendung von 10% CP oder 3,6% HP hatte keinen signifikanten Einfluss auf die Rauhigkeit. Die Schmelz-Mikrohärte war nach der Applikation von 10% HP (p = 0,0002) und 35% CP (p = 0,01) signifikant höher als bei der Kontrolle.

Der Vergleich der Anwendung von CP und HP zeigte nur geringe quantitative und qualitative Unterschiede. Das Bleichen der Zähne mit relativ hohen Bleichmittelkonzentrationen zeigte eine Zunahme der Schmelz-Rauhigkeit, hatte jedoch keinen negativen Einfluss auf die Mikrohärte. Die Bleichmittel mit niedrigen Konzentrationen hatten weder einen Einfluss auf die Rauhigkeit noch auf die Mikrohärte des Zahnschmelzes.

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Liste der Veröffentlichungen


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