Effects of 10% Carbamide Peroxide on Fracture Toughness and Microhardness of Human dentin In Situ

By

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A thesis submitted in conformity with the requirements for the degree of Masters of Science in Periodontology
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Abstract
There have been some reported negative effects on dental hard tissues associated with tooth bleaching. This in situ study evaluated the effect of 10% carbamide peroxide dental bleach on the dentin fracture toughness and microhardness. Compact tension fracture toughness dentin specimens, were prepared from extracted molars, irradiated and fitted into custom-made bleaching trays. The bleaching trays were loaded with either bleach (10% Carbamide Peroxide gel, Opalescence, Ultradent, n=34) or placebo gel (control group, n=31) and worn overnight for approximately 14 nights. Dentin specimens were tested 24-48 hrs after the end of treatment. The mean values for dentin fracture toughness were: 2.22 ± 0.71 & 2.26 ± 0.86 (MPa m $^{1/2}$) and for microhardness were: 66.80 ± 40.14 & 53.35± 42.52 (KHN) for bleaching and control respectively. Independent t-test showed no significant difference in dentin fracture toughness and microhardness after 14 days of in situ bleaching using 10% carbamide peroxide.
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I would like to dedicate my thesis to my son, Kian, who has lightened up my life with his presence and his kind heart. His sense of humour has made me to remember how to laugh at the end of a tough day.

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1. Introduction

Bleaching vital teeth has become a routine procedure in most general dental practices. This concept received a major stimulus with the introduction of carbamide peroxide at-home bleaching, about 20 years ago. Although there was controversy about the efficacy and safety of this concept during its initial years, worldwide use of carbamide peroxide and millions of applications have validated its use. (Christensen, 1998)

Tooth whitening materials are available as gels to be delivered in strips or in trays either in office or over the counter kits. The main active ingredient in tooth bleaching is hydrogen peroxide or carbamide peroxide. Hydrogen peroxide is a highly reactive chemical containing hydrogen and oxygen. Carbamide peroxide is a chemical that is made of hydrogen peroxide and urea. Beside their widespread use in cosmetic products, highly diluted hydrogen peroxide can also be used in disinfecting contact lenses. The decomposition products of carbamide peroxide, water, oxygen, ammonia and carbon dioxide are easily found in the normal processes of the human body. The Food and Drug Administration classifies carbamide peroxide as safe and effective for human uses as an oral antiseptic in 10% concentration. (Haywood, 1993) (Haywood & Robinson, 1997)

Bleaching agents affect lightening of tooth structure through decomposition of peroxides into free radicals. The free radicals break down large pigmented molecules in enamel and dentin into smaller, less pigmented molecules. This is the point (the saturation point) at which whitening should be terminated. If a prolonged period of exposure occurs, that is when patients want their teeth “over whitened”, the protein matrix breakdown can occur in enamel and dentin. (Goldstein, 1995)

With the increase in popularity and use of hydrogen peroxide based bleaching materials, there has been an increasing interest in determining the effects of hydrogen peroxide on the properties and characteristics of tooth structures. There is much more literature on enamel than on dentin. Tooth bleaching treatments have been associated with possible negative effects on dental hard tissues, including decreased bonding ability, (K. Titley, Torneck, & Smith, 1988a) (Torneck,

The dentin organic phase, the matrix, determines dentin morphology and is believed to be instrumental in the formation of the mineral phase. A fibrous web of collagen type I dominates the organic matrix. Minor amounts of other collagen types may also be present. The non-collagenous protein constitutes about 10% of the matrix (Linde, 1989). Some lipid containing components are also found in dentin. Dentin has an important role as the structural foundation of the tooth and in supporting the highly mineralized and brittle enamel. Acidity due to hydrogen peroxide exposure has been shown to remove dentin’s mineral components, leaving the exposed dentinal collagen vulnerable to hydrogen peroxide oxidation and leading to possible collagen denaturation (Jiang et al., 2007)

The structural integrity of the teeth is determined by mechanical properties such as modulus, strength and fracture toughness. It was reported that the flexural strength and modulus of bovine dentin decreased after an in vitro direct daily application of carbamide peroxide. (L. E. Tam, Abdool, & El-Badrawy, 2005) Significant reductions in tensile and shear strengths of dentin were reported after an in vitro direct intra coronal bleach application of 30% hydrogen peroxide. (Chng, Palamara, & Messer, 2002) Studies of direct applications of bleach to dentin are relevant because vital dentin can be exposed to direct bleach application clinically. It is impossible to avoid direct contact of bleach to the exposed dentin in the areas of occlusal attrition and root recessions during a typical home bleaching treatment using a tray or strips. It has been shown in another in vitro study that the fracture toughness of dentin was significantly reduced by the indirect application of peroxide bleaching agents (through intact enamel) as well as by a direct bleach application method to dentin, and it was reduced more for the longer application time period (8 vs. 2-weeks) and for the higher 16% (vs. 10%) bleach concentration. (L. E. Tam & Noroozi, 2007) The clinical relevance of these in vitro studies has been questioned. In situ or in
*vivo* studies are needed to determine whether the observed *in vitro* effects have practical clinical implications regarding tooth structural durability.

The adverse effects of dental bleaching on dental hard tissues are controversial. This thesis explores the effect of carbamide peroxide on dentin fracture toughness, microhardness and changes in dentin surface morphology by Scanning Electron Microscopy *in situ*. The possible association between changes in dentin fracture toughness and microhardness and changes in tooth color and sensitivity are also assessed. Background information on the substrate of interest (dentin) and the treatment of interest (bleaching) followed by a literature review related to dentin mechanical properties and to bleach effects, will lead in to the methodology of this study, the report of the *in situ* results and the discussion sections.

### 2. Literature Review

#### 2.1 Dentin

Dentin is the most abundant mineralized tissue and the main component in the human tooth structure. Dentin serves as an energy-absorbing cushion for constantly grinding enamel and for protection of the pulp. (Kinney, Marshall, & Marshall, 2003; Kruzic, Nalla, Kinney, & Ritchie, 2005) (Yan, Taskonak, & Mecholsky, 2009) Similar in composition to bone, it is composed largely of type-I collagen fibrils and nanocrystalline apatite mineral. The apatite mineral is suggested to provide strength, whereas the collagen matrix provides toughness. (Marshall, Marshall, Kinney, & Balooch, 1997) (Marshall et al., 1997)

Most naturally mineralized load-bearing tissues are composed of type I collagen fibrils and a reinforcing nanocrystalline apatite mineral phase. The mineral phase is partitioned between two sites: intrafibrillar mineral, which is confined within or immediately adjacent to the gap zones of the collagen fibrils; and extrafibrillar mineral, which lies within the interstitial spaces separating the fibrils. (Landis, Hodgens, Arena, Song, & McEwen, 1996) The fraction of mineral that is extrafibrillar is not well-established, although small-angle neutron scattering in bone (Bonar,
Lees, & Mook, 1985) suggests that as much as 70-75% of the mineral may be extrafibrillar. The mechanical consequences of this partitioning are also unknown. (Pidaparti, Chandran, Takano, & Turner, 1996) (Kinney, Habelitz, Marshall, & Marshall, 2003)

The composition of dentin has been reviewed by Marshall (1993), who reported it as approximately 55 volume % mineral, 30 volume % organic material, and 15 volume % fluid. The mineral component is primarily a calcium-deficient carbonate apatite whose crystallite size is smaller than that of enamel and larger than that of bone or cementum. (LeGeros, 1991). The mineral crystallites are needle-like near the pulp; the shape continuously progresses to plate-like with proximity to the enamel (Kinney et al., 2001). The crystallite thickness, ~ 5 nm, is invariant with location.

The interpenetrating hydroxyapatite and collagen form the dentin tubules in a highly ordered structure that makes human dentin much tougher than hydroxyapatite and collagen alone. (Yan et al., 2009) The most striking microstructural feature is the dentinal tubule, cylindrical channels which are the paths taken by the odontoblasts during dentinogenesis (Kruzic et al., 2005) and course continuously from the dentin–enamel and cementum–enamel junctions to the pulp. (Nalla, Kinney, & Ritchie, 2003; Nalla, Kinney, Marshall, & Ritchie, 2004)

As the tubules converge on the pulp chamber, the surface area of the intertubular dentin decreases and the tubule density increases, from about 1.9 x 10^6 tubules/cm^2 at the DEJ to between 4.5 x 10^6 (Garberoglio R. Bränström M, 1976) and 6.5 x 10^6 tubules/cm^2 at the dentin-pulp border. Each tubule is an inverted, elongated cone, with its smallest diameter (0.8 μm) at the DEJ and its largest diameter (3.0 μm) at the pulp. Each tubule is lined with a thin sheath of hyper mineralized collagen- poor (peritubular) dentin forming the cylindrical fibre reinforcement. (Eick, Gwinnett, Pashley, & Robinson, 1997) (Mjör, 1979)

The mineralized collagen fibrils, roughly 50-100 nm in diameter, are arranged orthogonal to the tubules, forming a planar, felt-like structure called the intertubular dentin matrix. This highly oriented microstructure is believed to confer anisotropy to the mechanical properties, although the magnitude and orientation of the anisotropy is not well established. (Nalla, Kinney, &
Located between the exterior enamel and the interior pulp, from a biological perspective, the dentin matrix can be described as a semipermeable barrier between enamel and pulp. From a microstructural perspective, the collagen fibrils in dentin serve as a scaffold for mineral crystallites that reinforce the matrix, supporting the surrounding enamel. From a biomechanical perspective, in brief, the mineralized dentin matrix preserves tooth function by helping to prevent propagation of cracks from the brittle enamel through the dentin-enamel junction into dentin (Imbeni, Kruzic, Marshall, Marshall, & Ritchie, 2005), thus preventing crown fracture. (Bertassoni, Habelitz, Kinney, Marshall, & Marshall, 2009). The inorganic mineral in dentin is believed to provide the strength and stiffness, and the organic collagen to provide the toughness. (Kinney, Marshall, & Marshall, 2003)

2.2 Mechanical Properties of Dentin

As early as the end of the 19th century, G.V. Black realized the importance of characterizing the mechanical properties of hard tooth tissues to enable setting requirements on the properties of restorative materials. Dentin is a biologic composite material composed of collagen fibrils and apatite crystals with highly mineralized peritubular dentin and the less mineralized intertubular dentin. (Iwamoto & Ruse, 2003) Dentin properties are similar to the surrounding mineralized tissues, e.g. cementum and bone, thus reducing stress concentrations at the interfaces during deformation. (Bertassoni, Habelitz, Kinney, Marshall, & Marshall, 2009)

The mechanical performance of the dentin is of major significance to the overall function of teeth. (Bertassoni, Habelitz, Kinney, Marshall, & Marshall, 2009) Therefore, knowledge of its mechanical properties is essential for predicting the effects of microstructural alterations resulting from the effects of the wide variety of dental procedures. (Kinney, Marshall, & Marshall, 2003) The mechanical properties of dentin describe the response of the tooth to applied loads, and allow for predictions of tooth strength and fracture properties. (Iwamoto & Ruse, 2003)
Basic mechanical properties including fracture toughness, microhardness, modulus of elasticity, shear modulus, tensile strength, compressive strength, proportional limit, and Poisson’s ratio for both enamel and dentin have been determined. However, due to the complexity of the structures involved and the challenges raised by the small samples available, evaluating the physical properties of dentin is a challenging task. (Iwamoto & Ruse, 2003)

There are many sources of variation that can affect dentin mechanical properties, these include:

- Location: Regional differences within a tooth due to the:
  - Orientation of dentin tubules and collagen fibrils surrounding the tubules relative to the load
  - Distance from DEJ or pulp
  - Mineral concentration
  - Tubules density
  - Peritubular vs intertubular dentin
- Level of calcification
- Hydration and Dehydration
- Age
- Storage media
- Sterilization technique

Enamel and dentin can best be considered as brittle materials with anisotropic fracture properties. Enamel is highly anisotropic; with the weakest path of fracture parallel to the enamel rods. Dentin is less anisotropic, with easiest fracture perpendicular to the dentinal tubules. (Rasmussen, Patchin, Scott, & Heuer, 1976) Many studies have shown that the orientation of the tubules affect dentin properties. (Rasmussen et al., 1976)(Rasmussen, Patchin, Scott, & Heuer, 1976) Rasmussen in 1976 estimated the work of fracture of human dentin and found that dentin is anisotropic with the easiest fracture direction perpendicular to the tubules. Carvalho studied the ultimate tensile strength (UTS) of human dentin and found that the UTS for the specimens with tubules parallel to and perpendicular to the plane of maximum normal stress were 80.0 and 57.6 MPa, respectively. Iwamoto and Ruse used a notchless triangular prism method to study the fracture toughness ($K_{IC}$) of human dentin. They found that $K_{IC}$ for the fracture plane parallel to
tubules was much greater than $K_{IC}$ for the fracture plane perpendicular to tubules ($\sim 2:0 \sim 1:1 \text{ MPa m}^{1/2}$). The superior mechanical properties of dentin along the plane parallel to the tubules are due to the direction of collagen alignment, which is perpendicular to the axis of tubules and acts to resist crack propagation. (Rasmussen, Patchin, Scott, & Heuer, 1976); (Iwamoto & Ruse, 2003); (Kinney, Marshall, & Marshall, 2003) (Yan et al., 2009)

Hardness and modulus of dentin generally increase in proportion to the mineral concentration and distance from the DEJ. The mineral concentration in normal dentin is relatively constant from the DEJ inward about 1 mm ($44.4 \text{ V\%}, \text{ SD} = 1.6$), at which point it begins to decrease gradually toward the pulp. In an approximately 0.3 mm layer surrounding the pulp, there is a sudden drop in mineral concentration to $35.0 \text{ V\% (SD} = 1.8$). (Kinney et al., 2003) (Featherstone, ten Cate, Shariati, & Arends, 1983)

Hardness falls precipitously in the inner layer of dentin of about 0.5 mm thickness that surrounds the pulp ($KH \sim 30 \text{ kg/mm}^2$). However, whether this is a result of increased porosity, or whether the intertubular dentin matrix is less mineralized, is an important question. In a careful and methodical study, Pashley and Parham (1985) determined that there was a significant correlation between decreased hardness and increased density of tubule lumens. The authors concluded that the reduced hardness was an end result of the lower mineral concentration brought about by the increased tubule porosity. This conclusion, however, was seriously challenged in a later study by Kinney et al (1996), who showed that most, if not all, of the decreased hardness near the pulp could be explained by a decrease in the hardness of the intertubular dentin matrix. Thus, it is likely that the intertubular dentin matrix near the pulp is less mineralized. (D. Pashley, Okabe, & Parham, 1985; Smith & Cooper, 1971) (Kinney, Marshall, & Marshall, 2003)

Kinney identified significant differences between peritubular and intertubular dentin with respect to their elastic moduli and hardness. He reported that the hardness and Young’s modulus of peritubular dentin was significantly higher than that of intertubular dentin. (Kinney, Balooch, Marshall, & Marshall, 1999) (Kinney, Balooch, Marshall, Marshall, & Weihs, 1996) (Iwamoto & Ruse, 2003)
Dentin is a vital tissue that can be changed by physiological and pathological conditions. The decay and attrition of teeth bring about changes in dentin calcification and morphological characterization. Studies seem to suggest that there are at least two forms of transparent dentin, a form associated with caries and a form associated with age-related changes in the root. Each type of transparency may exhibit unique biomechanical signatures. In a study by Senawongse et al., wear led to the formation of reactionary dentin and transparent dentin, decreased tubule diameters and tubular occlusion. These effects decreased the dentin permeability. (Stanley, Pereira, Spiegel, Broom, & Schultz, 1983)(Mendis & Darling, 1979)(Senawongse P. Otsuki M. Tagami J. Mjör I, 2006)

Little is known about the biomechanical of altered forms of dentin. It is absolutely essential that the properties of these altered forms of dentin be obtained before an understanding of how dentin pathologies or dental procedures affect tooth strength can be developed. (Kinney, Marshall, & Marshall, 2003)

To determine the influence of dentin hydration on the work of fracture of dentin, Kahler et al., conducted a controlled fracture toughness testing on bovine teeth. Significant differences were observed between the fracture toughness of hydrated and dehydrated dentine (554+/-27.7 J/m$^2$ vs 113+/-17.8 J/m$^2$). Observations of the crack tip region during crack extension revealed that extensive ligament formation occurred behind the crack tip. These ligaments provided considerable stability to the crack by significantly increasing the work of fracture, thereby acting as a fracture-toughening mechanism. Micro-cracking, reported as a fracture-toughening mechanism in bone, was also clearly seen. Only hydrated specimens showed a zone of in-elastic deformation, a region about the crack tip that appeared to suck water into the structure and to exude water behind the crack tip. In dehydrated dentin, no in-elastic zone was observed. Micro-cracking was present though the cracks were smaller, straighter and with less opening than hydrated dentine. Only limited ligament formation just behind the crack tip was observed. These differences resulted in a significantly lower work of fracture with unstable brittle fracture characteristics for dehydrated dentin. (Kahler, Swain, & Moule, 2003), (Kishen & Vedantam, 2007)
Some studies have shown that aging significantly decreases both the flexural strength and strain to fracture of dentin while others such as Moscovich in 1999 reported that the dentin hardness appears to be independent of the age and sex. Based on differences in the mechanical behaviour and microscopic features of the fracture surfaces from the young and old specimens, aging appears to result in an increase in both the rate of damage initiation and propagation in dentin. (Moscovich, 1999) (Figure 2.1 Arola & Reprogel, 2005)

Fig 2.1: A: Young dentin (24 year old male) with the fractured peritubular cuffs is highlighted by the arrows. Also note the recession of the peritubular cuffs with respect to the plane of the surrounding intertubular dentin. B: Old dentin (76 year old female). Note the absence of fractured cuffs in comparison to young dentin as well as the consistency in fracture surface of the intertubular and peritubular components. (Arola & Reprogel, 2005)

The storage methods of the extracted teeth, for dentin research can affect dentin mechanical properties. Storage method varies with regard to length of time and storage medium. Different solutions used for storage, such as water, saline, formaline, sodium azide in saline, 3% sodium hypochlorite and thymol, are noted in the literature. The reported effects of different disinfection and sterilization procedures on the tooth properties, such as dentin permeability or the effect on bond strength were assessed and in an attempt to standardize the storage solution for all dentin research, the use of 1% aqueous chloramine was recommended since it has no adverse effect on collagen of the dentin. (DeWald, 1997)
For tooth sterilization prior to biomaterials testing, gamma radiation can be used. It is superior to other sterilizing techniques (eg; chemiclave, autoclave, ethylene oxide, etc) and does not significantly alter the mechanical properties of dentin. A single gamma-irradiation dose of 25.5 KGy appeared to have no short term effects on the hardness of human coronal dentin and its permeability. The dose was chosen as it is the standard commonly used irradiation dose for medical sterilization by tissue banks for sterilization of orthopaedic allografts. It has been shown that a single ionization dose is sufficient to inactivate all tested bacteria, fungi, spores and viruses (notably M. tuberculosis, E. coli, S. aureus, model HIV and model Hep C) while preserving the biophysical integrity of the tissue. Gamma irradiation can preferentially inactivate viral and prion without excessive damage to albumin structure. (J. M. White, Goodis, Marshall, & Marshall, 1994) (Miekka et al., 2003) (Grieb et al., 2005) (Moscovich, Creugers, Jansen, & Wolke, 1999)

2.3 Fracture Properties of Dentin

Many of our conceptions of basic biomechanical properties of dentin have changed since the last comprehensive review by Waters in 1980. For example the concept of strength as an important engineering quantity has been suggested to be replaced by a fracture mechanics approach to dentin failure, since pre-existing flaws can cause teeth to fail at stresses far less than their theoretical strength. In particular, a lifetime modeling, or damage-tolerant approach appears promising for the development of a clinical predictor of tooth failure. These paradigm shifts have been facilitated by advances in measurement science combined with a better understanding of dentin microstructure. (Kinney, Marshall, & Marshall, 2003) As fracture is a critically important issue with human teeth, an understanding of the structural performance of dentin is crucial. (Kruzic et al., 2005)

2.3.1 Fracture Toughness

Resistance to fracture is a critically important issue with teeth. Tooth fracture is a significant dental problem that can necessitate restorative, endodontic and aesthetic treatment. In cases of severe tooth fracture, extraction may be the only treatment option. The incidence of tooth
Fracture was reported to be 20.5 per 1000 person per year (Fennis et al., 2002) and it is expected that the prevalence of tooth fracture will increase as the population retains their teeth longer. Whereas cusp fractures are common in posterior teeth, the anterior teeth are more susceptible to fracture at the gingiva, severing the crown of the tooth. Although such fractures have not been studied extensively, it is generally believed that they are catastrophic events induced by fatigue brought on by occlusal stresses. (Imbeni, Nalla, Bosi, Kinney, & Ritchie, 2003)

In teeth, restoration line angles serve as effective stress risers and are often the site of fracture initiation. In light of this, a stress-based fracture mechanics approach can provide a sound basis for the prediction of failure in human teeth. Under linear elastic conditions, an essential feature of this approach is that unstable fracture will occur when the stress intensity developed ahead of the tip of a pre-existing flaw exceeds the fracture toughness, $K_{IC}$, of the material. (Imbeni et al., 2003)

For relatively brittle materials such as calcified tissue, resistance to fracture is best quantified in terms of fracture mechanics parameters.

The powerful theoretical and experimental approaches of fracture mechanics have been extensively applied to engineering materials. The application of fracture mechanics principles to dental biomaterials is invaluable in evaluating structural integrity and in failure analysis. (Mecholsky, 1995) There are limited quantitative data on the toughness properties of human teeth. Although it has been suggested that a fracture mechanics approach would be more appropriate than a strength-of-materials approach for the study of tooth failure, surprisingly few studies have taken this approach. Fracture mechanics has gained more and more importance in analyzing the fracture of mineralized tissues, especially in the past two decades. (El Mowafy & Watts, 1986);(Imbeni et al., 2003; Iwamoto & Ruse, 2003);(Nalla, Kinney, Marshall, & Ritchie, 2004) The main reason is that mineralized tissues are generally complex in structure and require more elaborate methods to analyze how they fail.
Important exceptions to an otherwise-absence of quantitative fracture data are the works by Rasmussen et al, El Mowafy and Watts, and Imbeni et al. (Rasmussen et al., 1976; Rasmussen, 1984; Rasmussen and Patchin, 1984)(Kinney, Marshall, & Marshall, 2003).(Yan et al., 2009). (Ewalds and Wanhill, 1984; Kinloch and Young, 1983). (El Mowafy & Watts, 1986)

Rasmussen et al used a “work of fracture” defined as the work per unit area to generate new crack surface to quantify the toughness. Rasmussen et al determined the work-of-fracture of normal dentin in directions parallel and perpendicular to the tubule axis. These authors reported an influence of orientation on the toughness of dentin in that their measured work of fracture was lower for fracture perpendicular to the dentinal tubular direction compared with fracture in the plane of the tubules.

In addition to this work-of-fracture parameter, there are two other parameters which are regarded as of central importance in linear elastic fracture mechanics. These are the plane strain fracture toughness (\(K_{IC}\)) and the critical strain energy release rate (\(G_{IC}\)). These quantities have been extensively measured for bone and are considered intrinsic material properties. (Behiri & Bonfield, 1984) (El Mowafy & Watts, 1986) The use of the fracture toughness (\(K_{IC}\)) test better quantifies the tooth’s resistance to fracture than conventional strength tests such as the flexural strength test. (Kelly, 1995) Plane-strain \(K_{IC}\) values are true measures of structural integrity that do not change with different specimen geometries or loading conditions.

El Mowafy and Watts, utilized fracture mechanics based measurements, specifically using compact-tension specimens to measure intrinsic fracture toughness in dentin. Using an orientation parallel to the long axis of the tubules, these authors reported a mean \(K_{IC}\) value of 3.08 MPa√m (standard deviation 0.33 MPa√m) for dentin, which was found to remain constant over the temperature range 0° C – 60° C. (Imbeni et al., 2003)

Plane strain fracture toughness (\(K_{IC}\)) and critical strain energy release rate (\(G_{IC}\)) were calculated using the following equations:

\[
K_{IC} = \frac{PY_2}{BW^{1/2}}
\]
\[ G_{IC} = \frac{K_{IC}^{2}}{E} \]

P = maximum load required to fracture specimen

\[ Y_2 = f \left( \frac{a}{W} \right) \]; a tabulated function of effective notch length, \( a/W \); (British Standard 5447; 1977)

B = Specimen thickness

W = Specimen net width

E = Modulus of elasticity of the material under condition of test

Values of the modulus of elasticity of dentin used to calculate \( G_{IC} \) at the three test temperatures were 15.20, 13.26, and 11.15 GN/m\(^2\) at 0°C, 37°C, and 60°C, respectively. These values were representative of a statistically significant (\( p < 0.01 \)) variation of modulus with temperature for dentin. (El Mowafy, 1984)

The work by El Mowafy and Watts (1986) was the first attempt to measure the intrinsic fracture toughness, \( K_{IC} \), of dentin with ASTM standard specimen geometry: the compact tension specimen. Based upon an unpublished value of the yield stress, the authors assumed that the test resulted in a valid measure of \( K_{IC} \). They could therefore equate their fracture toughness measures with the energy release rate, \( G \), and obtained good agreement with the earlier data of Rasmussen. (Rasmussen et al., 1976; Rasmussen, 1984; Rasmussen and Patchin, 1984)

Three valuable findings came from the work of El Mowafy and Watts (1986):

1. Compact tension specimens could be fabricated from coronal dentin;
2. Fracture toughness was high for a brittle material, indicating that either the collagen fibrils or the tubule lumens provide a toughening mechanism;
3. Fracture toughness was independent of temperature through a range from 0°C to 60°C.

However, since the fracture plane was parallel to the tubules (i.e., in the orientation associated with the highest work of fracture), it is possible that the fracture toughness could be considerably lower in the orthogonal plane. (Kinney, Marshall, & Marshall, 2003)(Imbeni et al., 2003)(Imbeni et al., 2003)
2.3.2 Hardness

While strength is of great importance in determining a material’s service performance, bulk properties are only part of the story. The surface behaviour of a material is relevant to its ability to withstand the stress. (Darvell, 2009) Hardness is a relevant property; since wear resistance is proportional to tissue hardness. There are many different methods of measuring hardness, but usually it is an indication of resistance to deformation caused by penetrating, scratching or bouncing an object on test surfaces of various degrees of polish. Microhardness tests on dentin measure the resistance of the dentin to deformation caused by penetration of an indenting stylus. (Kinney, Marshall, & Marshall, 2003)

Due to the large size of the indentation in relation to the dentin microstructure, the microindentation technique provides only a composite average of the various structural microcomponents present in dentin. (Chng, Ramli, Yap, & Lim, 2005) (Mair, Stolarski, Vowles, & Lloyd, 1996)

Indentation hardness is determined by applying an indenter of specified geometry to the surface under a predetermined load and from a measurement of the indentation or its depth, its area is calculated. It corresponds to the stress that material could just support at equilibrium without further deformation, that is to say a kind of yield point. Therefore, the smaller the indentation, the greater the hardness will be. (Darvell, 2009)

Knoop hardness (KH) was developed by at the National Bureau of Standards (now NIST) in 1939. The indenter used is a rhombic-based pyramidal diamond that produces an elongated diamond shaped indent. Knoop tests are mainly done at test forces from 10g to 1000g, so a high powered microscope is necessary to measure the indent size. Because of this, Knoop tests have mainly been known as microhardness tests. The newer standards more accurately use the term microindentation tests. The magnifications required to measure Knoop indents dictate a highly polished test surface.
Fig 2.2: Knoop testing is done with a rhombic-based pyramidal diamond indenter that forms an elongated diamond shaped indent. The indenter is pressed into the sample by an accurately controlled test force and is maintained for a specific dwell time, normally 10 - 20 seconds. After the dwell time is complete, the indenter is removed leaving an elongated diamond shaped indent in the sample. The size of the indent is determined optically by measuring the longest diagonal of the diamond shaped indent.

Although the microhardness test does not provide specific information on changes that occur within a substance or material, it can provide indirect evidence of mineral loss or gain in the dental hard tissues and is commonly used in experiments involving demineralization and remineralization. Previous investigations have shown the suitability and practicality of using Knoop and Vicker’s microhardness test for evaluating surface changes of dental hard tissues treated with chemical agents, and the Knoop method is the most popular. (Arends & ten Bosch, 1992) (Attin, Hannig, Wiegand, & Attin, 2004) (Lewinstein & Grajower, 1981) (Gedalia, Ionat-Bendat, Ben-Mosheh, & Shapira, 1991; Lewinstein, Hirschfeld, Stabholz, & Rotstein, 1994; Seghi & Denry, 1992; Unlu, Cobankara, Altinoz, & Ozer, 2004) (Murchison,Charlton, & Moore, 1992) (Meredith, Setchell, & Swanson, 1997) (Magalhães CS. Moreira AN. Campos WR. Rossi FM. Castilho GA. Ferreira RC, 2006)

Since its introduction, the Knoop (Knoop et al., 1939) indenter has proved to be the workhorse in studies of dentin. The long aspect ratio (7.11 times longer in one dimension) allows for accurate
measurements of area, even with shallow indentations. The Knoop microhardness test, therefore, is extremely useful for the small, thin specimens typical of studies of mineralized tissues.

Hardness is defined in units of pressure, or force per unit area of indentation. Unlike Vickers, which uses the contact area of the indenter stylus, the Knoop method uses the projected area, \( A_p \), in the calculation of hardness (KHN):

\[
KHN = \frac{P}{A_p} = 14.22 \frac{P}{l^2}
\]

Where \( P \) is the applied load (in kg) and \( l \) is the length (in mm) across the long axis of the remnant impression. (Kinney, Marshall, & Marshall, 2003)

Many investigators have determined that dentin hardness depends on mineral concentration. In addition to its association with the mineral concentration, hardness has been correlated with location in the tooth due to the differences in dentinal tubule density at different locations and significant differences between peritubular and intertubular dentin with respect to their elastic moduli and hardness. According to Chng et al, the location of dentin in terms of buccal, lingual, mesial or distal had no effect on micro hardness of dentin, but in each group, the mean KHN was higher for outer dentin than inner dentin. (Kinney et al., 1999) (Chng, Yap, Wattanapayungkul, & Sim, 2004)

In the literature the average hardness value for enamel and dentin has been reported in the range from 270 to 350 KHN (or from 250 to 360 VHN) and from 50 to 70 KHN respectively, depending on the load applied (usually between 10 and 100 g). Knoop (KHN) and Vickers (VHN) hardness tests have reported approximately the same value. (Lewinstein, Hirschfeld, Stabholz, & Rotstein, 1994) (Rodrígues JA. Basting RT. Serra MC. Rodrígues Júnior AL, 2001) & (Pinheiro Júnior EC. Fidel RA. da Cruz Filho AM. Silva RG. Pécora JD, 1996) (Renne, 2009)
2.4 Bleaching

Aesthetic dentistry, particularly tooth whitening, has increased dramatically in recent years. Bleaching by the application of hydrogen peroxide or carbamide peroxide to enamel is a popular method of whitening vital teeth. According to a Clinical Research Associates (CRA) survey, 62% of the dentists prescribe at-home carbamide peroxide bleaching. (Lewinstein, Fuhrer, Churaru, & Cardash, 2004)(Christensen, 1998; Unlu et al., 2004) Various bleaching techniques have been used over the years to obtain whiter teeth. Early methods included chloride of lime and soda (Dwinelle, 1850), nitric acid (Fitch, 1861), and sulphurous acid. (White, 1861) Hydrogen peroxide (H₂O₂) was introduced as an irrigant for disinfecting alveolar abscesses and necrotic teeth. (Harlan, 1882) At that time, it was suggested that it also might be useful as a bleaching agent for discolored teeth. (Hanks, Fat, Wataha, & Corcoran, 1993)

Since the introduction of night-guard vital bleaching in 1989 by Haywood and Heymann, a myriad of professional and mass market tooth whitening products have become available. These products most commonly contain hydrogen peroxide or carbamide peroxide as the active agent. They are effective, versatile and available in a range of formulations, concentrations and activation modes. (Barghi, 1998) Carbamide peroxide is an alternative source of hydrogen peroxide because it is readily dissociated into urea and hydrogen peroxide on contact with water. Several in vitro and in vivo studies have demonstrated the efficacy of external bleaching solutions with various concentrations of either hydrogen peroxide or carbamide peroxide as the primary active ingredient. Most studies showed a relatively greater increase in tooth lightness by either increasing concentration of the applied gels or by using longer duration of bleaching treatment times. (Kugel, Petkevis, Gurgan, & Doherty, 2007)

Bleaching methods can be broadly divided into internal or external bleaching. In internal bleaching, bleaching agents are placed in the pulp chamber, in direct contact with dentin. In external bleaching, bleaching agents are applied to the enamel surface. External bleaching may be performed at the dental office (in-office bleaching) or by applying the agent in a gel form within the confines of a custom tray or strip by the patient (home bleaching). (Lewinstein et al., 2004) (Unlu et al., 2004)(Christensen, 1998)
The ‘in-office’ tooth bleaching technique requires the use of 30–35% hydrogen peroxide and direct monitoring by the dentist. The use of high-intensity light, for raising the temperature of the hydrogen peroxide and accelerating the rate of chemical bleaching of teeth was reported in 1918 by Abbot. (Bleaching techniques in restorative dentistry: An illustrated guide 2001) Other approaches for heating the peroxide have historically been described to accelerate tooth bleaching, such as heated dental instruments. The direct application of heat soon fell out of favour, because of evidence suggesting that it may cause cervical resorption. (Ontiveros & Paravina, 2009) Excessive heating can also cause irreversible damage to the dental pulp. (Zach & Cohen, 1965)

Contemporary approaches for in-office bleaching have focussed on accelerating peroxide bleaching with simultaneous illumination of teeth with various sources having a range of wavelengths and spectral power, for example, halogen curing lights, plasma arc lamps, lasers and light-emitting diodes. (Sulieman, 2005) For some light sources, significant increases in pulpal temperatures have been measured using in vitro models during tooth bleaching. (Eldeniz, Usumez, Usumez, & Ozturk, 2005) Techniques using chemical catalysts, such as manganese chloride or catalase, to accelerate the peroxide bleaching effect avoid the necessity for expensive lights as well as radiant heating effects from the light. However, clinical studies investigating the use of supplementary light or chemical catalysts on the effectiveness of vital bleaching have been equivocal. (Ontiveros & Paravina, 2009)

The ‘at-home’ night guard vital tooth bleaching technique has come into the focus of both the dentist’s and the patient’s interest, since the technique allows for patient application of bleaching agents outside the dental office. The typical at-home technique uses a 10% –15% carbamide peroxide solution in a custom-made bleach tray worn at the patient’s convenience for one to eight hours daily for several weeks. It is an effective treatment system for mildly stained teeth, has a high level of patient and practitioner acceptance, involves simple procedures, takes up minimal dental office time, is economical, and causes minimal discomfort when compared with chair-side procedures. The main disadvantages of night guard vital bleaching are poor patient compliance, soft-tissue irritation from overextended trays and tooth sensitivity. Sore throat,
laxative effects and occlusal problems also have been reported in the literature. The active agent in carbamide peroxide bleaching gel is available in bleaching trays for more than 10 hours. After two hours, more than 50% of the active agent is available, and 10% is available after 10 hours. (Matis, Gaiao, Blackman, Schultz, & Eckert, 1999)

Most bleaching agents are strong oxidizing agents and although they are highly effective in lightening tooth color, concern has been expressed regarding their use, especially hydrogen peroxide, in relation to associated post-bleaching complications. These include the aforementioned soft tissue effects and tooth sensitivity, as well as adverse tooth effects such as alteration in the surface morphology of dentin, (K. Titley, Torneck, & Smith, 1988) change in chemical composition of dentin,(Rotstein, Dankner, Goldman, Heling, Stabholz, & Zalkind, 1996) increased dentine permeability (Heling, Parson, & Rotstein, 1995) and external cervical root resorption. (Harrington & Natkin, 1979)(Madison & Walton, 1990) Bleaching has also been associated with changes in the biomechanical properties of dentin. (Chng et al., 2005) (Unlu et al., 2004)

Data concerning the side-effects of bleaching agents on the dental hard tissues is rather limited and not yet clearly established. It is important to test the effect of the bleaching materials on all dental hard tissues but the effects of whitening on human dentin are of special interest as dentin is both targeted by bleaching agents and supports the highly mineralized and brittle external enamel crown.

### 2.5 Effects of bleaching on tooth physical and mechanical properties

The chemical properties of carbamide peroxide are of great advantage for the clinical whitening of natural teeth. However, the possible interaction of the peroxide radical with the organic or inorganic components of tooth could lead to some changes in the physical and mechanical properties of the enamel and dentin. The small size of the molecule allows it to diffuse into the interprismatic space of the semipermeable enamel structure and penetrate into dentin. (Seghi &
The bleaching agent also may come directly into contact with exposed dentin in the areas of caries, enamel defects or abrasion.

The issue of the effects of bleaching on the tooth structure is controversial. Generally, effects of carbamide peroxide bleaching are considered no worse than those resulting from routinely used commercial soft drinks or other dental procedures. (Haywood, 1992) Some studies described conflicting changes of the enamel and dentin while others indicated that short-term regimens of 10 or 15% carbamide peroxide did not significantly affect enamel and dentin. In spite of the widespread use of bleaching, there is no general agreement as to the effect of bleaching agents on enamel and dentin. If performed under the careful guidance of a dentist, at-home whitening is a reliable treatment. However, further investigation of the clinical significance of this process is needed. (Unlu et al., 2004)

The specific concerns for enamel and dentin when using a bleaching solution relate to:

1) Chemical changes, erosion and abrasion resistance
2) Surface morphology and hardness
3) Strength and fracture toughness
4) External root resorption
5) Bond strength
6) Tooth sensitivity

2.5.1 Chemical Changes, Erosion and Abrasion Resistance

The exact mechanism by which bleaching agents affect dentin has yet to be fully elucidated. Some studies have indicated that hydrogen peroxide caused dissolution of inorganic material, a reduction in calcium-phosphorus ratio, and a reduction in the organic components of dentin by protein oxidation. Bleaching materials may adversely affect the dental hard tissues by changing the original ratio between the organic and inorganic components of the tissues and increasing their solubility. (Rotstein, Dankner, Goldman, Heling, Stabholz, & Zalkind, 1996b) (Chng et al., 2005)(Mair et al., 1996)
Covington examined surface compositional changes in enamel and dentin after exposure *in vitro* to a bleaching solution for 45 days. Although dentin and enamel showed a slight decrease in calcium and phosphorous after bleaching, the authors concluded there are no radical alterations in the composition of enamel and dentin. (Unlu et al., 2004) McCracken and Haywood measured the amount of calcium lost from enamel exposed to a 10% carbamide peroxide solution. They suggested that the amount of calcium loss was small and may not be clinically significant.

Studies have shown that changes such as erosion, porosity, and loss of mineral substance in the surface morphology of enamel and dentin occur following bleaching. (Ben-Amar et al., 1995) (Bitter & Sanders, 1993) Azrak *et al* demonstrated in an *in vitro* study that bleaching agents with a high concentration of peroxide or an acidic pH can influence the surface roughness of sound or eroded enamel. (Azrak, Callaway, Kurth, & Willershausen, 2010)

Pretty *et al* conducted an *in vitro* study to determine if enamel that had been bleached by carbamide peroxide gel (10%-16%-22%) was at increased risk of either acid erosion or demineralization than unbleached enamel. Following statistical analysis there were no differences detected between the bleached and unbleached areas, nor between the different concentrations of the bleaching solution. They concluded that tooth bleaching with carbamide peroxide does not increase the susceptibility of enamel to acid erosion or caries. (Pretty, Edgar, & Higham, 2005)

Engle used specimens of human enamel and root dentin in an *in vitro* study and randomly divided them into eight groups that underwent 10% carbamide peroxide bleaching, erosion and dentifrice abrasion. His results showed that dental erosion and the more abrasive dentifrice increased tooth brushing wear on enamel surfaces, while bleaching showed no deleterious effect. Dentin wear also increased after subjection to erosion and use of the more abrasive dentifrice. Bleaching increased surface loss on non-eroded dentin and decreased loss on eroded dentin when it was brushed with the less abrasive dentifrice. (Engle, Hara, Matis, Eckert, & Zero, 2010)
Although the detrimental effects of erosion/abrasion and bleaching have been investigated, there are limited published data on the association among the processes. (Engle et al., 2010) Extrapolation of *in vitro* results to clinical situations is also limited. (Azrak et al., 2010)

### 2.5.2 Surface Morphology, Surface Hardness

In the past decade, numerous studies evaluated the effects of peroxide-containing bleaching agents on tooth hard tissues with more focus on enamel. Haywood *et al* reported that 10% carbamide peroxide, applied to simulate 5 weeks of exposure, did not alter surface morphology, etch or decalcify enamel. Another study by Murchison *et al* demonstrated that short term regimens of 10% carbamide peroxide did not significantly affect the hardness of the enamel surface. Oltu and Gurgan demonstrated that 10 or 16% carbamide peroxide did not seem to affect the structure of enamel, whereas 35% carbamide did affect the structure, and recommended the use of lower concentrations of carbamide peroxide. Unlu *et al* investigated the effects of home bleaching agents that contain carbamide peroxide in different concentrations such as 10% and 15% on the surface hardness of human enamel and dentin. There were no statistically significant differences between the untreated control specimens and the specimens treated with the bleaching materials for enamel and dentin at any given measurement time. (Unlu et al., 2004)

Arcari *et al* in an *in situ* study evaluated the influence of two home-use tooth bleaching regimes (1 hour/day and 7 hours/day) using 10% carbamide peroxide on the surface microhardness of dentin over a 21-day period. The results demonstrated that the difference between the 1-hour and 7-hour groups was not significant. However, the 7-hour group, when compared with the control group, demonstrated statistically significant differences with a reduction in microhardness. (Arcari, Baratieri, Maia, & De Freitas, 2005)

In an investigation of the effects of hydrogen peroxide on bovine teeth, Al-Salehi *et al* reported that ion release from both enamel and dentine increased with increasing hydrogen peroxide concentration and they also concluded that microhardness of enamel decreased significantly with bleaching. (Al-Salehi, Wood, & Hatton, 2007) A decrease in microhardness of human dentin following application of bleaching agents has also been demonstrated. (Pécora JD. Cruzfilho AM. Sousaneto MD. Silva RG, 1994)
Office bleaching uses a higher concentration of the bleaching agent applied for shorter periods of time than home bleaching and may affect the hard dental tissues differently. Lewinstein et al evaluated the effect of different concentrations of two “in-office bleaching” and two “home bleaching” agents applied for different time periods (5, 15, or 35 minutes) on the hardness of human enamel and dentin. Significant decreases in KHN of enamel and dentin were found after bleaching for all test groups, dependent on the accumulated bleaching time. Opalescence (10% carbamide peroxide) group showed an 18% reduction in enamel and 13% in dentin microhardness. They concluded that the “in-office” bleaching technique reduced the hardness significantly more than the “home bleaching” technique. (Lewinstein et al., 2004)

It has been proposed that the presence of a remineralizing agent, fluoride or saliva, inhibits demineralization or a decrease in microhardness values. (Featherstone, Cutress, Rodgers, & Dennison, 1982) (Featherstone et al., 1982) Therefore, reported decreases in microhardness results in vitro may not be relevant to the clinical situation where fluoride and saliva play a role. The in situ or in vivo use of bleaching agents on tooth hard tissues requires further analysis. (Unlu et al., 2004)

De Freitas et al used human dentin slabs (3x3 mm) to evaluate the effect of six bleaching agents: Nite White (NW) 10% and 22% Excel 2Z (Discus Dental), Rembrandt (REM) 10% and 22% (DenMat), Opalescence 10% and 20% (Ultradent) and a placebo agent on microhardness of demineralized dentin at different time intervals. The result demonstrated that Opalescence 10% and 20% increased dentin microhardness in different magnitudes, whereas REM 10% and 22% which induced mineral loss during application, showed microhardness recovery in the post-treatment period for 14 days with artificial saliva. (de Freitas, Turssi, Hara, & Serra, 2004) This result was consistent with the author’s previous study with the similar design to evaluate the microhardness of human dentin exposed to two 10% carbamide peroxide agents. (de Freitas et al., 2002)

Basting et al fixed enamel and dentin fragments on molars intraorally for 2 periods of 3 weeks and bleached the specimens with 10% carbamide peroxide (Opalescence) and then with a placebo. A significant reduction of 10%- 23% KHN was recorded for bleached enamel but the
KHN for dentin was unaltered. (Basting, Rodrigues Junior, & Serra, 2001) Araujo and colleagues in an in situ study (to simulate the natural action of saliva/oral fluids on the enamel specimens) demonstrated no significant decrease in microhardness (KHN) of human enamel after 21 days of bleaching with 10% carbamide peroxide. (Araujo, Baratieri, Vieira, & Ritter, 2003) (Matis, 2004)

**2.5.3 Strength and Fracture Toughness**

Chng et al conducted an in vitro experiment in an attempt to answer the question of whether intracoronal bleaching weakens coronal tooth structure by lowering the ultimate tensile strength (UTS), micro punch shear strength (MPS), and microhardness of dentin. They concluded that although intracoronal bleaching on its own may weaken dentin only to a small extent, coupled with the excessive loss of tooth structure from previous decay and endodontic treatment, it may be enough to tip the balance and cause the tooth to fracture in function. (Chng et al., 2002)

An in vitro study by Tam et al showed a significant decrease in flexural strength and modulus of bovine dentin after direct bleach application, while an indirect application of 10% carbamide peroxide did not significantly decrease dentin strength and stiffness. (L. E. Tam et al., 2005)

As an extension of the group’s previous work, they evaluated the effects of the prolonged exposure to peroxide bleaching agents (8 weeks) on the fracture toughness of human dentin in another in vitro experiment. The results showed a significant decrease in dentin $K_I$ with direct and indirect bleach application after 8 weeks but it was greater for the direct application method. The last study was more clinically relevant because it was done using human dentin. The authors pointed out that the relative contribution of changes in either the collagen or non-collagenous proteins of dentin or the mineral phase to the reductions in $K_I$ of dentin needed further study. (D. H. Pashley, 2007) (L. E. Tam & Noroozi, 2007)

In an in vitro study on the effect of hydrogen peroxide on fracture toughness, mineral loss and microhardness of bovine teeth, Seghi et al reported an almost 30% reduction in the enamel fracture toughness and a significant decrease in abrasion resistance after bleaching for a period of 12 hours with 10% carbamide peroxide. They commented that this behaviour was most likely
due to an alteration of the organic matrix of enamel under the chemical action of hydrogen peroxide.

The clinical consequences of these in vitro observations are unknown since factors, such as buffering capacity of the dentin and the remineralizing effect of saliva should be considered. It is possible that the intrinsic mechanical property of fracture toughness is not affected by the same factors that affect surface microhardness. The effects of hydrogen peroxide solution on dentin in vivo or in situ may be less pronounced than that observed in vitro studies. Further clinical studies should be conducted to examine the influence of these factors on the fracture toughness, hardness and erosion susceptibility of dentin when exposed to hydrogen peroxide. (Chng et al., 2005)

### 2.5.4 Bond Strength

Problems regarding reduced resin bonding to bleached enamel have been found since 1988. (K. C. Titley, Torneck, Smith, & Adibfar, 1988). The resin bond strength to recently-bleached enamel was reduced to approximately 60–67% of its original value, depending on the types and concentrations of the bleaching agents used. (Unlu, Cobankara, & Ozer, 2008) Different experimental conditions, including bleaching period, elapsed time, and adhesive agents, were found to affect the adhesion of resin components to bleached teeth. (Unlu et al., 2008)(Sung, Chan, Mito, & Caputo, 1999)

The reduced bond strength was generally attributed to the presence of peroxide-related substances in the surface and subsurface enamel that remained after bleach treatment. Oxygen decomposed from residual peroxide resides in the resin–enamel interface, thus impairing the adhesive attachment and resin tag extension. (K. C. Titley, Torneck, Smith, Chernecky, & Adibfar, 1991) Studies have confirmed that residual oxygen inhibited the polymerization of the adhesive and resin tag extension. (K. C. Titley, Torneck, Ruse, & Krmc, 1993)(Dishman, Covey, & Baughan, 1994) Although the entrapped oxygen is considered to be the main cause of the inferior bond quality, the structural defects on enamel have also been claimed as responsible for these changes. (Chuang, Chen, Chang, & Liu, 2009) The bleaching agents caused
morphological alterations, including porosity and loss of prismatic form on enamel, which weakened the enamel near the adhesion interface and indirectly diminished the bond strength. Perdigao et al similarly attributed the impaired bonding to the structural changes of surface and subsurface enamel, such as the loss of mineral and alterations in the organic substance. (Perdigao, Francci, Swift, Ambrose, & Lopes, 1998)

The reduced ability to bond to bleached enamel takes time to reverse to a level comparable to unbleached enamel, consequently delaying related restorative or orthodontic treatments. (Haywood, 1996) Studies investigating the appropriate time point for bonding of composites to enamel after termination of in office bleaching with 25–35% hydrogen peroxide reported that bond strength returned to normal values when the composite was applied on the specimens 24 h or 2 weeks after bleaching. (Attin et al., 2004)

The presence of additional oxygen in the tooth may alter the optical properties of the tooth too. Because of this phenomenon, as well as the inhibiting effects of oxygen on bond strengths of composite, shade selection for restorative materials and bonding is best delayed for 1 to 2 weeks after completion of bleaching. (Haywood, 1996)

2.5.5 External Root Resorption

Hydrogen peroxide used for non-vital tooth bleaching has been associated with the development of external root resorption. (Harrington & Natkin, 1979) (Rotstein et al., 1991) Heithersay reported intracoronal bleaching as a sole and associated predisposing factor for External Cervical Resorption (ECR) in 3.9% and 13.6% of cases, respectively. (Heithersay, 1999) This pathological condition was reported in clinical cases as well as in experimental animal studies. The exact mechanism of bleaching-induced root resorption has not been fully established. Rotstein et al demonstrated that the presence of cemental defects at the cemento-enamel junction could result in hydrogen peroxide from the pulp chamber of root-filled teeth escaping to the external tooth surface via dentinal tubules during intracoronal bleaching with 30% hydrogen peroxide. (Madison & Walton, 1990) (Rotstein et al., 1991) (Rotstein, Lehr, & Gedalia, 1992)
It has been suggested that hydrogen peroxide might denature dentin and provoke an immunologic response. (Cvek & Lindvall, 1985) In addition, the pH at the root surface of teeth is reduced to about 6.5 by intracoronal placement of a “walking bleach” paste, this slightly acidic environment is known to enhance osteoclastic activity, which might result in external root resorption. (Patel & Ford, 2007) (Harrington & Natkin, 1979) (Rotstein et al., 1992)

In direct, non-vital bleaching, it was suggested that the effect of hydrogen peroxide on the organic and inorganic components increased the risk of its leakage from the pulp chamber to the periodontal ligament which may cause external root resorption. It has been recommended that the use of high concentrations of hydrogen peroxide for non-vital bleaching purposes should be limited. (Lewinstein, Hirschfeld, Stabholz, & Rotstein, 1994) Vital tooth bleaching treatments have not been associated with external cervical resorption.

### 2.6 Color Change

Aesthetics of the teeth are of great importance to patients, including tooth color. In a survey of 3215 subjects from the United Kingdom 50% perceived they had some kind of tooth discoloration and in the USA, 34% of an adult population were dissatisfied with their current tooth color. (Odioso, Gibb, & Gerlach, 2000) Discoloration can be caused by several factors including aging, hereditary conditions, chemical damage to the teeth, trauma, consumption of staining food and beverages such as coffee, tea, soft drinks, medications and smoking.

The color of the teeth is influenced by a combination of their intrinsic color and the presence of any extrinsic stains that may form on the tooth surface. (Joiner, 2004) Intrinsic tooth color is associated with the light scattering and adsorption properties of the enamel and dentin, with the properties of dentin playing a major role in determining the overall tooth color. (ten Bosch & Coops, 1995) (Meireles, Demarco, dos Santos Ida, Dumith Sde, & Bona, 2008)

The tooth whitening mode of action involves diffusion of the hydrogen peroxide through enamel to the enamel–dentin junctions and dentin where it oxidizes colored pigments to a lighter color. Carbamide peroxide dissociates into hydrogen peroxide and urea when in contact with soft
tissues or saliva at oral temperatures. Peroxide can diffuse through enamel and dentin because of its low molecular weight. Whereas hydrogen peroxide further degrades into oxygen and water, urea degrades into ammonia and carbon dioxide. The mechanism of the action of hydrogen peroxide is thought to be related to its ability to form oxygen free radicals that interact with adsorbed colored organic molecules. The subsequent oxidation of colored macromolecules and pigment stains, produce smaller and lighter molecules that cause a shift of the visible absorption spectrum from a longer to a shorter wavelength, resulting in colorless or less dark compounds and whitening action. (Kugel et al., 2007)

The rate at which teeth lighten during bleaching varies considerably among patients. In general, there is a gradual lightening in color that eventually reaches a plateau of maximum lightening for that patient. Beyond this point, further treatment makes no change. Where this color plateaus is located and how long it takes to get there varies from patient to patient. Some patients’ teeth lighten quickly and progress to a very light shade, whereas other patients’ teeth take a longer time to obtain either the same or a more moderate result. There are no predictors for rate or final outcome. Just as each patient responds differently, so do different teeth. Since most of the tooth color comes from the dentin, in places where the dentin is thicker, the color change will be slower. (Haywood, 1996)

The rate of chemical reactions can be increased by increasing the temperature. A light source can be used to heat or “activate” peroxide to accelerate the chemical redox reactions of the bleaching process. (Sun, 2000) The light source can also energise the tooth stain to aid the overall acceleration of the bleaching process. (Smigel, 1996) Some products that are used in light activated bleaching procedures contain ingredients that claim to aid the energy transfer from the light to the peroxide gel and are often colored materials, for examples, carotene and manganese sulphate. (Sulieman, 2005) The efficacy of light activated systems versus non-light activated controls in clinical studies is limited and conflicting. (Joiner, 2004)

The duration of the bleaching treatment depends on the method of bleaching used. With the at home bleaching method, it has been reported that nine of ten patients experiencing a lightening of their teeth in 2 to 6 weeks application time. (Haywood, 1996) Trays are usually worn one to
eight hours during the day or night for 2 weeks. The duration of application for in-office bleaching agents varies from 10 to 20 minutes for 1 to 3 sessions.

Two of the key factors in determining overall tooth whitening efficacy by peroxide containing products are concentration and time. Sulieman et al compared the in vitro tooth bleaching efficacy of gels containing 5% to 35% hydrogen peroxide and found that the higher the concentration, the lower the number of gel applications required to produce uniform bleaching. (Sulieman, Addy, MacDonald, & Rees, 2004) In general, higher concentrations are faster than lower concentrations. However, lower concentrations can approach the efficacy of higher concentrations with extended treatment times. Leonard et al compared the in vitro tooth bleaching efficacy of 5%, 10% and 16% carbamide peroxide gels and found the whitening was initially faster for the 16% and 10% that of 5% concentration. (Leonard, Sharma, & Haywood, 1998) However, the efficacy of the 5% approached the higher concentrations when the treatment time was extended. (R. H. Leonard, Sharma, & Haywood, 1998) (Joiner, 2004)

Other factors which can influence tooth bleaching outcome include initial tooth color, type of stain, and subject’s age. (Joiner, 2004) The darker the teeth at baseline, the longer it can take to lighten them. (Mahony, Barker, Engel, & Walden, 2003) In addition, it was reported that when the tetracycline discoloration is located in the neck of the tooth, the prognosis for bleaching is the poorest; when it is dark gray or blue, the prognosis also is poor. (Haywood, 2000a) Bleaching works more efficiently for teeth with a yellow hue. (Ishikawa-Nagai, Terui, Ishibashi, Weber, & Ferguson, 2004) An analysis of the clinical results, with over 600 subjects undergoing tooth bleaching, indicated that the yellower the teeth at baseline, the greater the magnitude of the whitening response. (Gerlach & Zhou, 2001)

The type of intrinsic stain can play a significant part in the ultimate outcome of tooth bleaching. Mild to moderate tetracycline staining tends to respond to extended bleaching regimes of 2–6 months, but it is documented that severe tetracycline staining is more difficult to bleach. (Haywood, 2000a) Matis et al demonstrated that when using a tray delivery technique, tetracycline-stained teeth can be effectively lightened with the extended use of tooth whiteners (6
months), more than 55% of tooth lightening occurred within 1 month. (Matis, Wang, Eckert, Cochran, & Jiang, 2006)

There is also a relationship between subject age and the magnitude of whitening response, with younger subjects experiencing greater tooth whitening. Further, there was a relationship between subject age and the initial color and the magnitude of whitening response. Older subjects with less yellow initial tooth color exhibited the smallest mean color change post bleaching, whereas younger subjects with more yellow initial tooth color exhibited the greatest mean color change post bleaching. (Gerlach & Zhou, 2001) Neither gender nor coffee/tea consumption had any significant effect on the tooth whitening response. (Qualtrough & Burke, 1994)

After bleaching, there is a relapse in color in the first few days followed by stabilization of the tooth color. (Haywood, 1996) Previous reports have noted that at 1.5 years post treatment, 74% of teeth that have been bleached have retained their lightening without any additional treatment. At 3 years post treatment, 62% still retained a clinically acceptable lightening. (Haywood, Leonard, Nelson, & Brunson, 1994) A 2-week time span is needed for the color of the whitened teeth to stabilize prior to placing permanent restorations.

A number of approaches are available for measuring changes in tooth color. These include visual measurements by trained clinicians and instrumental measurements using spectrophotometry, Chroma-meters and digital image analysis. (Joiner, 2004) Recently, digital systems, spectrophotometers (Braun, Jepsen, & Krause, 2007), colorimeters (Joiner, 2004) or digital cameras (Wee, Lindsey, Kuo, & Johnston, 2006) have been used to measure tooth color. Within these systems, color is expressed in CIEL^*a^*^b^* space, which provides its specification in three dimensions and allows for more accurate assessments. (Meireles et al., 2008)

Visual assessment with a shade guide is still the most common method used for color classification in longitudinal studies involving bleaching agents. (Kihn, Barnes, Romberg, & Peterson, 2000; Matis, Mousa, Cochran, & Eckert, 2000)
With the continued interest in tooth whitening amongst basic and clinical researchers, the further mechanistic understanding and optimisation of the factors controlling the tooth whitening process will continue to expand. This will give further improvements to the tooth whitening products and procedures, and give significant benefits to the field of aesthetic dentistry.

2.7 Tooth Sensitivity

A commonly reported potential side effect of home-use whitening systems is tooth sensitivity. The incidence and severity of tooth sensitivity during at-home bleaching varies and is reported in the range of 9% to 100% but more commonly is in the 60% range. (L. Tam, 2001) Tooth sensitivity usually is mild and transient, but occasionally may cause significant discomfort. Mild tooth sensitivity can be expected in approximately one-half of patients who undergo home whitening treatment using 15% carbamide peroxide and 0.11% fluoride ion. Approximately 10% of patients may experience moderate sensitivity, and 4% of patients may experience severe sensitivity for one to two weeks. (Jorgensen & Carroll, 2002)

Hydrogen peroxide, alone or with heat, caused pulpal inflammatory changes which was reversible after 60 days. (Seale, McIntosh, & Taylor, 1981) There have been reports in the literature of diffusion of hydrogen peroxide through intact enamel. (Bowles & Ugwuneri, 1987) Peroxide can diffuse through enamel and dentin into the pulp chamber because of its low molecular weight and cause reversible pulpitis and tooth sensitivity. (Kugel et al., 2007)

The amount of hydrogen peroxide that diffuses through the dentin is most dependent on its original concentration within the bleaching agent and the length of time the agent comes into contact with the dentin. Bleaching agents with higher osmolarity might not allow diffusion at the same rate as those with lower osmolarity, since the higher osmolarity would tend to draw fluid away from the pulp. The osmolarity appeared to be controlled by the gel composition rather than by the peroxide concentration. (Hanks et al., 1993)(L. Tam, 2001)
The hydrodynamic theory is the primary explanation for dentin sensitivity cases such as root recession. However, the primary cause of tooth sensitivity related to bleaching is attributed to reversible pulpitis brought about from the ingress of hydrogen peroxide in the pulp. Pulpal inflammation lowers the pain threshold. Tooth sensitivity is a multi-faceted condition: dentin may be exposed and permeable, but the host response is also important and must include inflammatory components. It should be kept in mind that the degree of initial, localized pulpal inflammation beneath open permeable tubules probably plays a role in determining both the initial level of hypersensitivity and the potential for resolution of symptoms. Pulpal degeneration and the amount of secondary dentin may also influence the response to treatment. (Camps & Pashley, 2003)

The possible risk factors and causes of tooth sensitivity include the patient’s inherent sensitivity, the pH of the bleaching agent, and the concentration of the active bleaching ingredient, the daily frequency of bleach application and the gingival recession. Reducing the tooth sensitivity improves patient compliance and comfort throughout the treatment. Although the tooth sensitivity that occurs during at-home vital tooth bleaching has not been associated with pulpal problems after the cessation of bleaching, it can affect patient compliance. (R. H. Leonard Jr, Haywood, & Phillips, 1997)

A common recommendation for the treatment of tooth sensitivity associated with tooth whitening is to reduce the frequency or duration of bleaching applications. Another approach is applying topical fluorides or desensitizing pastes. Tam demonstrated that the use of carbamide peroxide gel containing potassium nitrate and fluoride produced less tooth sensitivity during a 2-week at home bleaching treatment than a carbamide peroxide gel without potassium nitrate and fluoride. (L. Tam, 2001) Both potassium nitrate and fluoridated materials have been used to treat the problem of dentin hypersensitivity not related to tooth bleaching. It has been suggested that potassium nitrate reduces tooth hypersensitivity by preventing nerve repolarization after initial depolarization, thereby reducing pulpal or dentinal sensory nerve activity. (Markowitz & Kim, 1990; Touyz & Stern, 1999) Browning et al conducted a double-blind, placebo-controlled clinical trial to provide evidence that the addition of low levels of potassium nitrate and/or potassium nitrate and fluoride significantly reduced postoperative sensitivity relative to products
that did not contain either agent. Their results showed that the addition of a small percentage of potassium nitrate to a 10% carbamide peroxide tooth whitener significantly reduced postoperative sensitivity without reducing efficacy. (Browning et al., 2008)

Patients considering home whitening treatment should be advised that tooth sensitivity is a common side effect and that their effects generally are mild and transient. (Jorgensen & Carroll, 2002)(L. Tam, 2001) If gingival recession is present, the probability of tooth sensitivity increases. The patient should be advised to use desensitizing toothpaste before the start of bleach treatment, and higher hydrogen peroxide concentrations and more frequent bleaching should be avoided. However, if tooth whitening procedures are performed properly, tooth sensitivity effects generally are mild and transient. (Jorgensen & Carroll, 2002)(L. Tam, 2001)

2.8 Factors Affecting Adverse Bleaching Effects on Enamel and Dentin Physical and Mechanical Properties

**Duration of bleaching:** It is uncertain whether there is a potential for cumulative damage resulting from prolonged or multiple exposures to dental bleach. The results of some of the *in vitro* studies suggest that there is an association between the length of bleaching time and dentin fracture toughness, and a relatively progressive reduction in dentin fracture toughness would be expected over the course of 336 hours of bleaching. The point at which the reduction in dentin fracture toughness becomes clinically significant is unknown and would depend on many factors. (Woo, Ho, & Tam, 2010)

**Concentration:** It has been shown in literature that the adverse effects of the bleaching agents on enamel and dentin are dose related. Pecora *et al* verified a decrease in human dentin microhardness after application of the bleaching agents with various concentrations for 72 hours with 30% hydrogen peroxide causing the greatest decreases in dentin microhardness. (Pécora JD. Cruzfilho AM. Sousaneto MD. Silva RG, 1994) The effect of hydrogen peroxide concentration on mineral loss and microhardness of bovine teeth was investigated in an *in vitro* study using bovine dentin and 0%, 3%, 10% or 30% hydrogen peroxide solutions for 24h at 37°C. The
results showed that ion release from both enamel and dentin increased with increasing hydrogen peroxide concentration. (Al-Salehi et al., 2007) It has been suggested that the use of high concentrations of hydrogen peroxide for bleaching purposes should be limited. (Lewinstein, Hirschfeld, Stabholz, & Rotstein, 1994)

However another study on the effect of highly concentrated (38%) bleaching agents on microhardness and surface roughness of bovine enamel and root dentin revealed that bleaching did not alter the enamel microhardness and surface roughness, but in root dentin, microhardness was affected by the bleaching agent used. (Faraoni-Romano, Da Silveira, Turssi, & Serra, 2008)

Tam et al also reported that the fracture toughness was reduced more for the higher 16% (vs. 10%) bleach concentration. (Tam and Noroozi, 2007) The clinical relevance of these in vitro studies has been questioned. In situ or in vivo studies are needed to determine whether the observed in vitro effects have practical clinical implications regarding tooth structural integrity. (L. E. Tam & Noroozi, 2007)

**Test conditions:** The majority of studies investigating the effects of bleach on enamel and dentin physical and mechanical properties are in vitro studies. The in vitro conditions will have a significant effect on the tooth substrate, which will be affected by loss of vitality and storage conditions that change the hydration, buffering capacity, and permeability of dentin. In vital teeth, there is an outward movement of fluid through dentinal tubules, which would tend to expel and buffer the applied bleach. In vitro conditions also do not replicate clinical bleach treatments. The oral cavity superimposes temperature changes; chemical changes, movement and salivary flow on bleach treatments in vivo, and bleaching agents are more subject to washout and degradation in the oral cavity than in vitro. There is a greater potential for the tooth to recover from adverse bleach effects in vivo than in vitro because the tooth is vital and/or is exposed to remineralizing solutions such as saliva in the oral cavity.

Studies have demonstrated tooth remineralization after bleach treatment. Potential therapies to prevent or reverse the weakening effect of prolonged exposure to bleaching agents have been explored. (D. H. Pashley, 2007) One in vitro approach to counteract the potential demineralizing effects of bleaching agents was to store the teeth in remineralizing solutions that mimicked the
ion products of calcium and phosphate in saliva. Another approach would be to add amorphous calcium phosphate to the bleaching gels. (D. H. Pashley, 2007) Some studies have shown that the use of a low-concentration fluoride solution can restore the hardness of bleached enamel and dentin. (Lewinstein et al., 2004)

Attin et al subjected bovine enamel specimens to 4 to 12 hour bleaching cycles with 10% carbamide peroxide (Opalescence) and 8-hour remineralization in artificial saliva. Specimens then were covered with fluoride varnish and, for a separate group, stored in 0.2% sodium fluoride solution 1 minute prior to remineralization. A third group of specimens was bleached but not fluoridated and a fourth group was not bleached and stored in distilled water. A 1.3% reduction in VHN was recorded in the fluoride varnish group, 2.9% in the NaF solution, 13% reduction in the no fluoride bleach group, and no reduction in the unbleached specimens. The bleached and unfluoridated specimens showed a significantly higher hardness loss compared to the fluoridated specimens, and no significant difference was observed between the two fluoridated bleached groups. It was concluded that remineralization of bleached enamel is improved by application of highly concentrated fluoride. (Lewinstein et al., 2004) (Attin, Kielbassa, Schwanenberg, & Hellwig, 1997)

De Freitas et al exposed dentin fragments to Opalescence and Rembrandt (10% carbamide peroxide) for 8 hours per day for 42 days and noted KHN reductions of 26% and 60%, respectively. The fragments were subsequently stored in artificial saliva, which restored the hardness to baseline after an additional 14 days. In clinical situations, continuous remineralization and rehydration effects of saliva plays an important role to protect the tooth structure integrity. The clinical relevance of reported in vitro adverse effects on enamel and dentin is uncertain. The authors are unaware of clinical tooth fractures that are attributable to bleach treatment. (L. E. Tam, Kuo, & Noroozi, 2007)
2.9 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) has been used in many studies to investigate the potential morphology changes to enamel and dentin produced by the bleaching agents. (Josey, Meyers, Romaniuk, & Symons, 1996)(K. Tittley, Torneck, & Smith, 1988b) (D. J. White, Kozak, Zoladz, Duschner, & Gotz, 2000) Josey et al demonstrated changes to the surface and subsurface layers of enamel after a one week vital bleaching procedure with 10% carbamide peroxide using scanning electron microscopy. (Josey et al., 1996) Ernst et al (Ernst et al., 1996) evaluated four bleaching agents using scanning electron microscopy and found that enamel underwent slight morphological alteration after bleaching. (Unlu et al., 2004)

On the other hand, some studies have shown no change in surface morphology of human enamel outside of normal variations using SEM evaluations. (White, Kozak, Zoladz, Duschner, & Gotz, 2000) Haywood et al (1990) reported no change in enamel surface morphology with 10% carbamide peroxide using mouthguard bleaching technique. (Haywood, Leech, Heymann, Crumpler, & Bruggers, 1990) Potocnik et al also showed that the local microstructural in enamel caused by 10% carbamide peroxide were likely not clinically significant. (Potocnik, Kosec, & Gaspersic, 2000)

Atomic Force Microscopic images of dentin post-bleaching have shown that both intertubular and peritubular dentin were affected by hydrogen peroxide, with peritubular dentin appearing more resistant to hydrogen peroxide than intertubular dentin. This is likely due to the difference in composition between intertubular dentin and peritubular dentin. Peritubular dentin is hypermineralized and lacks collagen as an organic component of its matrix. On the other hand, collagen is the major organic component of intertubular dentin, making up approximately 92% of the organic matrix. (Chng et al., 2005)

There are many more limited studies evaluating the same effects on the dentin. In the study by Tam et al for evaluating the prolonged effects of peroxide bleaches on dentin fracture toughness, scanning electron microscopy examination revealed some evidence of increased demineralization, particularly in the tubule walls, of bleached specimens but in general the
surface morphology of the dentin fracture surfaces of the bleach and control dentin groups appeared similar. (L. E. Tam & Noroozi, 2007) Scanning Electron Microscopy (SEM) is also used for fractography which is a standard technique for analyzing the fracture behaviour of brittle materials. (Mecholsky, 1995) (Yan, Clifton, Reep, & Mecholsky, 2006) The fracture surfaces of materials usually contain characteristic patterns that detail the fracture process. For most materials fractured in a brittle or quasi-brittle manner, these patterns allow researchers to identify the fracture origins. (Yan et al., 2009) In this study SEM is used to look for in situ dentin surface changes after 2-week at home bleaching with 10% carbamide peroxide when compared with the changes caused by the same treatment with the placebo gel.

3. Null Hypothesis

In situ bleach treatment has no effect on the mechanical properties of dentin.

4. Objectives

The purpose of the present investigation is:

1- To compare the fracture toughness and microhardness of dentin after either bleach or placebo treatment in order to elucidate the effects of tooth bleaching on human dentin mechanical properties in a clinical setting.

2- To determine if there is an association between changes in dentin fracture toughness/microhardness and changes in tooth color or tooth sensitivity.

3- To determine in situ effects of 10% carbamide peroxide bleaching gel on dentin surface morphology after at-home bleaching regimen by Scanning Electron Microscopy.
5. Materials and Methods

5.1 Specimen Preparation

Extracted (within past 3-6 months) third molar teeth were collected from patients with a non-contributory medical history. The adherent soft tissues were carefully removed from the teeth’s surfaces and the teeth were stored in a %1 Chloramine solution and kept refrigerated until sectioned. Teeth with visible signs of decay, fractures, or cracks were discarded.

Compact tension test specimens (n=65) as described by El Mowafy and Watts and nominally conforming to ASTM E399 (American Society for Testing and Materials, 2001) were prepared from dentin. (Figures 5.3 and 5.4) The location and orientation of the dentin utilized to form fracture toughness specimens were standardized. Dentin slices, approximately 1.6 mm thick, were cut from the coronal dentin of each tooth parallel to the occlusal surface, below the occlusal enamel while avoiding the pulp tissue. A water-cooled slow-speed diamond saw (Buehler Ltd., Lake Bluff, IL, USA) was used to cut the dentin slices and only one slice was obtained from one tooth. A constant cooling flow of water was provided at the cutting field and a linear cutting speed of 1 mm/min was used to minimize any temperature rise in the dentin during sectioning. The dentin slices then were cut to form the rectangular specimens with approximate dimensions of 4.5x4.6 mm.

![Specimen preparation, coronal dentin is sectioned using a slow-speed saw (Buehler). A central notch of specific length (a) was then made in each specimen with the diamond disc.](image)

(Yan et al., 2009) (El Mowafy & Watts, 1986)
Flex x929.7, [Abrasive Technology] Premier Products Co, Plymouth Meeting, PA, USA) and sharpened with a razor blade. This notch acted as a stress concentrator and crack propagation originated from this notch. The notch length was important since a/W ratio must be in the range 0.45 - 0.55, where a is the notch length and W is the net width of the specimen. The specimen dimensions were measured by means of a micrometer to make sure the a/W ratios lay within the acceptable range for the compact tension test method.

Finally, a tungsten carbide drill bit mounted on a bench drill was used to drill two cylindrical holes, 0.80 mm in diameter, in each specimen to provide means of attachment for tensile loading. The compact test specimen is a standard configuration used for $K_{IC}$ testing. The specimen preparation aspects of this study were time consuming and required great care.

- Total width(C) = 4.7mm
- Net width(W) = 3.75mm
- Height (H) = 4.50mm
- Thickness(B) = 2mm
- a/w ratio = 0.45-0.55 (±0.02)
- Effective notch length(a) = 0.25w to 0.40 w
- Notch width (N) ≤ 0.065 W
- Hole diameter(D) = 0.80 mm
- B/W ratio = 0.25-1.25

**Fig 5.4:** Compact tension test specimen geometry (Tam et al, 2007)
One surface of each specimen was polished with 800/1200/2400 grit silicon carbide paper in sequence for the Knoop microhardness testing while kept hydrated. All preparations were performed following standard infection control protocol. Then the specimens were stored in artificial saliva (Table 5.1) in individual containers and sent for gamma-irradiation for sterilization at 2.5MRad for 1500 minutes. The gamma radiation was done at the Southern Ontario Centre for Atmospheric Aerosol Research (SOCAAR) through the department of Chemical Engineering and Applied Chemistry at U of T. Dentin specimens then were stored refrigerated until needed for use.

5.2 Participants selection

The study protocol was approved by the University of Toronto, Office of Research Ethics (approval #24941). Sixty five adults over 18 years old with no allergy and a non-contributory dental history (ie. no xerostomia, no untreated carious lesions, not presently undergoing orthodontic treatment) were included in the study. Participants who had recently (within 1 year) bleached their teeth and current smokers were not included in the study.

The investigator was responsible to recruit participants, assign them to bleach and placebo groups based on whether or not the patient wanted to have his/her teeth whitened, explain the study and obtain the informed consent and dispense the selected material: placebo (Ultradent Products, Inc.), or bleach (10% carbamide peroxide, Opalescence, Ultradent products, South Jordan, UT, USA, Lot# B4YJ9), take the base line and final measurements and impressions, fabricate customized bleaching trays to hold the dentin specimen and collect the data. The investigator was not blind to the patient assignment when collecting the data. The composition of the materials used is shown in Table 5.1.
<table>
<thead>
<tr>
<th>Materials</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial Saliva (1L)</td>
<td>0.1 L each of 25mM K₂HPO₄, 24mM Na₂HPO₄, 150mM KHCO₃, 100mM NaCl, and 1.5mM MgCl₂, 0.006 L of 25mM citric acid, 0.1L of 15mM CaCl₂, pH adjusted to 6.7 with NaOH or HCl, 0.05% by weight thymol</td>
</tr>
<tr>
<td>10% carbamide peroxide gel</td>
<td>10% carbamide peroxide, glycerine, carbopol, water</td>
</tr>
<tr>
<td>Placebo gel (Ultradent)</td>
<td>glycerine, carbopol, water</td>
</tr>
</tbody>
</table>

Table 5.1: Materials used for the bleaching and the placebo groups.

5.3 Fabrication of custom-made bleaching trays with the dentin

Custom-made bleaching trays were provided for each patient to deliver the placebo or bleaching agents to the patient’s teeth as well as to hold the dentin specimen. The method for bleaching tray fabrication is described below and shown in Figures 5.5 and 5.6.

Alginate impressions were taken of each patient’s maxillary dental arch for fabrication of stone casts. Light Cured Block Out Resin (Ultradent) was applied to the facial surfaces of the incisors and premolars to be bleached to an approximate thickness of 0.5 mm. Small blocks of composite resin with the approximate dimensions of the actual specimens were made and one was bonded onto the buccal surface of a maxillary premolar to create a space for the actual dentin specimen in the bleaching tray. The bleaching trays (0.9 mm-thick, Sof-Tray Sheets, Ultradent were vacuum formed to the stone casts and trimmed along the gingival margins. A randomly selected dentin specimen was sutured to the space created in the tray (4-0 silk) on a maxillary premolar. The fit of the bleaching trays were checked to make sure there is no impinging on the soft tissue.
Fig 5.5: Light Cured Block Out Resin (Ultradent) was applied to the facial surfaces of the incisors and premolars. The resin block (replica of the specimen) was cured to the facial surface of one of the premolars to create a space for holding the dentin specimen in the bleaching tray.

Fig 5.6: The dentin specimen was secured to a custom-made bleach tray using a suture.

All participants received demonstration and instructions concerning the proper use of the bleaching agents. To standardize participants’ oral hygiene regimens, they were asked not to use dentifrices that contain whitening agents. The participants were asked to wear the bleaching trays, containing the dentin specimen, overnight for 14 nights to follow a typical at-home bleaching regimen. None of the participants had used desensitizing or whitening tooth pastes in the past 4 weeks. At the end of each daily bleaching treatment, the participants were asked to rinse the tray and dentin specimen with tap water to remove all external traces of bleach and
store the tray and dentin specimen in artificial saliva until the next bleach treatment. If the participant experienced severe tooth sensitivity, he/she was advised to skip one or two days of bleaching. Most participants easily completed the 14 nights of bleaching within an 18-day period. They were advised that the 14 nights of treatment should take place within a 28 day period. Participants were asked to make a daily record on a provided log form of: 1) date and the number of hours of the bleaching done, and 2) the degree of tooth sensitivity experienced on a 100mm visual analogue scale.

### 5.4 Sensitivity

Tooth sensitivity was additionally tested by before- and after-treatment cold testing. The cold testing involved the placement of 0.3mL 7°C glycerine gel to the middle of the facial surface of the lateral incisor for 3 seconds. It has been suggested that cold water at 7°C was ideal for the identification of sensitive teeth. (Gillam & Newman, 1993) At the end of 3 seconds, the gel was wiped off and the participant was asked to score the lateral incisor tooth sensitivity on a visual analogue scale. Each day during the active treatment of the study (bleaching or placebo) participants recorded their tooth sensitivity by marking it on a 100-mm visual analogue scale. The extremes of the line represented the limits of pain a patient might experience (no pain at one end and severe pain or discomfort at the other end of the line). Participants were asked to place a mark on the 100 mm line which indicated the intensity of their current level of sensitivity or discomfort during the treatment. The daily entries for tooth sensitivity were measured from the visual analogue scales to the nearest millimeter to obtain the daily visual analogue score for each patient. The average VAS per participant for the duration of the treatment was calculated (mean VAS) and then the average VAS for the group was calculated. (L. Tam, 2001)

<table>
<thead>
<tr>
<th>Patient #</th>
<th>DAILY LOG FORM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>Tray Wear</td>
</tr>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
5.5 Shade selection

5.5.1 Visual color matching

Visual color matching of the tooth to the shade tabs was performed before and after treatment for both bleaching and placebo groups. Visual color matching scores were recorded independently by the investigator and an experienced lab technician. Shade selection was carried out under similar clinical conditions for all participants. Shade tabs were matched to one maxillary central incisor, unless the centrals had any restorative coverage (extensive resin restorations, veneers or crowns, etc), in which case a laterals incisor was used. The visual evaluation was made by comparing the shade tabs with the middle third of the selected upper anterior incisor. Shade matching results were recorded. The 16 shade tabs in the guide were numbered from 16 (highest value – B1) to 1 (lowest value – C4) for statistical analysis. (Fig 5.7) (Meireles, Santos, Bona, & Demarco, 2010a)(Ontiveros & Paravina, 2009)(Browning et al., 2008)

The investigator used shade tabs with C4 (darkest shade) to B1 (brightest shade) range (Vita Classic shade guide). The Easyshade also provided tooth shade via using the 16 shade tabs in the Vita shade guide (C4- B1). The technician used two shade guides: Vita Classic and Vivadent-Ivoclar (Fig 5.8) with a 20-point scale (with 4 extra shades brighter than B1). For data analysis the shades were numbered according to their ranking from 1 (lowest value corresponds to C4 shade) to 16 (highest value corresponds to B1 shade). For correlation and reliability analysis in order to have a comparable data, tooth shades obtained by the technician were coded using a 16-point scale with shades brighter than B1(010, 020, 030, 040) as B1, all having rank of 16.

For each participant (placebo and bleaching group) tooth shades were measured by investigator, the technician and Spectrophotometer independently and measurements took place before and after the treatment. The difference between shade scores was calculated for each participant to determine the improvement in tooth color. Then the average score for color change for each group was obtained and the analysis was performed to investigate the difference in shade before and after treatment for each group. Shade of the specimens was not specifically measured.
**Fig 5.7:** Tab arrangement and numeric order for Vitapan Classical (“Value” scale)

**Fig 5.8:** Vita Classic Shade Guide and Vivadent-Ivocla

### 5.5.2 Instrumental method for color measurement

In our study, the instrumental color monitoring was performed before and after bleaching and placebo treatment using a contact-type intraoral spectrophotometer (Vita Easyshade, Vita Zahnfabrik, Bad Säckingen, Germany) with a 5mm diameter probe. The Easyshade has a spectral range 400–700 nm (with a wavelength resolution of 25 nm), and it can provide tooth shade assessment using the 16 shade tabs in the Vita shade guide (B1–C4) as well as the CIEL*a*b* color system. (Meireles, Santos, Bona, & Demarco, 2010) In our study we used the 16 shade tabs (Vita shade guide) to be comparable with other shade match readings. The spectrophotometer was calibrated according to manufacturer’s instruction prior to use.
A custom positioning jig was made for each subject’s maxillary arch to provide accurate repositioning and measurement on the middle third of labial surface of one of the central incisors. The jig was fabricated using clear self-cure resin. Using the gypsum molds of the maxillary arch, replicas of the spectrophotometer’s probe were made from a vinylpolysiloxane impression by using gypsum. Then replicas were attached to the gypsum molds using cyanoacrylate adhesive. The replica was positioned in the center of the middle one-third of the buccal surface of the selected incisor tooth. Acrylic resin was poured around the gypsum replicas on the labial surface and to the incisal edges. After the resin hardened, the custom jigs were separated and used as a guide for repeatable positioning of the spectrophotometer’s fiber-optic probe at a right angle to the tooth’s axis and at the center of the buccal surface. (Giachetti, Bertini, Bambi, Nieri, & Russo, 2010)

The Easyshade Compact LED illuminates the target area, independent of lighting conditions. The unit was turned on and calibrated prior to each use. The measuring probe which was covered with a disposable plastic sleeve and was placed on the tooth (made certain that the probe is flat on the tooth). By pressing the switch on the hand piece while holding it on the tooth, the shade was read on the unit and recorded by the investigator. (Fig 5.9)
5.6 Fracture Toughness Test

After the last bleaching and placebo treatment session, the dentin specimens and log forms were returned to the investigator. The dentin specimens were kept in artificial saliva and refrigerated until tested for fracture toughness (within 24-48 hours) using an Instron universal testing machine (Model 4301, Instron Corp, Canton, Ma, USA) and specially designed mounting jig. (Fig 5.10)

Fig 5.10: Fracture toughness testing. A tensile load was applied to the fracture toughness specimen mounted on the jig, in a universal mechanical tester (Instron) at a rate of 10mm/min until fracture to calculate the fracture toughness parameter ($K_{IC}$).

The specimen’s dimensions including (a-B-W-2H) were recorded to the nearest 0.01mm using a micrometer. The specimens were mounted on the jig with the help of round wire 0.5 mm diameter. Tensile loading (100 N load cell) was applied at a rate of 10 mm/min until specimen fracture. The force recorded at the fracture point was used to calculate $K_{IC}$ for all specimens.

Fractured dentin halves were stored in two separate containers: artificial saliva and 100% ethanol and later placed in the refrigerator.
5.7 Hardness Test

The fractured dentin halves, stored in artificial saliva, were used for the hardness test immediately after fracture toughness testing. The Knoop hardness number (KHN) was obtained in a Knoop microhardness tester (Tukon 300 DF- FM ) with a 100g static-load and 20 seconds dwell time. The specimens were mounted firmly on an aluminum stud using a hand presser (Leitz Wetzlar, Germany) keeping the polished surface of the specimen on top. Then the specimens were placed in the Knoop microhardness tester and at least three indentations were made on each specimen in the bleaching and placebo group. The length of the longest diagonal of the diamond shaped indent was measured with microscope and the KHN was calculated by the tester and was recorded for each indentation. (Fig 5.11) The mean KHN number for each specimen was obtained from all the indentations for that specimen.

Fig 5.11: A Knoop microhardness tester was used on the surface of the dentin fracture toughness specimen after fracture (100g/20 sec).
5.8 Scanning Electron Microscopy

The effects of the 10% carbamide peroxide (at-home regimen) on the surface morphology of the fracture surface of dentin specimens were observed and compared to the fracture surface of the placebo treated specimens. Five samples were randomly selected from each group (Bleaching and Placebo) for SEM examination.

The fractured specimens that were stored immediately after fracture in 100% ethanol were prepared for SEM examination after 2 weeks. These specimens were critical-point-dried (Polaron CPD-7501, Fisons Instruments, Hertfordshire, England) using ethanol and liquid CO₂ as the intermediate and final dehydration fluids. The critical point method of drying helps avoid the deformation and collapse of vulnerable dentin surface structures resulting from surface tension effects by never allowing a liquid/gas interface to develop. The specimens were then mounted on a 12-mm aluminum stub using a cyanoacrylate adhesive, sputter coated with platinum (Polaron SC515 SEM Coating Systems, Fisons Instruments, England) and observed using a scanning electron microscope (Hitachi S-2500, Hitachi, Tokyo, Japan) at x 1000 magnification.
6. Sample Size Analysis

The minimum sample size was determined using power analysis tool (G*Power 3.1.2) with assumption of type I error = 0.05, standard deviation = 0.8 MPa*m$^{1/2}$ for fracture toughness, desired power 0.80 and a smallest difference of interest between placebo and bleaching groups = 0.7 MPa*m$^{1/2}$. The minimum sample size was determined to be 22 participants in each group (total sample of 44). We increased the sample size to compensate for possible uncertainties such as participant exclusion or withdrawals. Our final total sample was total of 65 participants. For our sample of 65, the power is expected to be 0.93. (Fig 6.12 A)

Sample size calculation for the microhardness test with assumption of type I error = 0.05, standard deviation = 15 KHN (generally obtained in previous studies), desired power (0.80) and a smallest difference of interest between placebo and bleaching groups=10 KHN resulted in the minimum sample size of 29 participants in each group (total sample of 58). The graph below shows relationship between total sample size and statistical power of the test. For our sample of 65, the power is expected to be about 0.85. (Fig 6.12 B)

![Graph A](image1.png)

![Graph B](image2.png)

**Fig 6.12:** The graph shows relationship between total sample size and statistical power for fracture toughness test (A) and microhardness test (B).
7. Statistical Analysis

For each of the tests, the mean, standard deviation and standard error were calculated. Dentin fracture toughness and microhardness were examined using independent samples t-test. Reliability analysis was performed to examine the internal degree of consistency between tooth shades measurements obtained by Investigator, Technician and the intraoral Spectrophotometer (Vita Easyshade).

Correlation analysis was used to examine the pairwise strength of relationship between measurements and the significance of the correlation coefficient was determined using t-test. Improvement in tooth color was explored using non-parametric Wilcoxon Signed Ranks Test. Levels of tooth sensitivity were compared between participants in bleaching and placebo groups using independent samples t-test (for mean daily sensitivity scores), paired-samples t-test (for mean change in sensitivity), and Chi-square test (for categorical levels of sensitivity).

Correlation analysis was performed to explore the relationships between change in dentin fracture toughness, microhardness and tooth sensitivity and change in tooth color separately for bleaching and placebo groups. Level of significance = 0.05 was used for all statistical tests.
8. Results

All subjects in this study completed the course of treatment. The average daily treatment time was 85.2 hours for the bleaching group and 82.2 for the placebo group. Bleaching duration was between 9 and 17 days with average being 13.52 days. The mean daily bleaching treatment time was 6.44 hours (SD=1.38). The bleaching group included 34 participants: 23 Female and 11 Male, with an average age of 27.26 years (SD: 6.45, range: 19-39) and the Placebo group included 31 participants: 17 Female and 14 Male, with an average age of 29.35 (SD: 7.89, range: 21-55).

8.1 Analysis of Dentin Fracture Toughness (K\text{IC})

One sample in the placebo group was excluded from the fracture toughness data analysis due to the improper fracture path of the specimen during testing. Shade of the specimens was not specifically measured but the lighter color in the bleaching group specimens was obvious.

Independent samples t-test revealed no statistically significant difference in mean dentin fracture toughness between bleaching ($M = 2.22, SD = .71, SE = .12, n = 34$) and placebo ($M = 2.26, SD = .86, SE = .16, n = 30$) groups, $t (62) = .231, p = .818$. The results for dentin fracture toughness for bleaching and placebo groups are shown in Figure 8.13.

Fracture toughness ranged between 0.90 – 3.91 MPam$^{1/2}$ in bleaching group and between 1.05 – 4.20 MPam$^{1/2}$ in placebo group. (Fig 8.14)
**Fig 8.13:** Fracture toughness ($K_{IC}$) of human dentin after *in situ* application of 10% carbamide peroxide and placebo gel, using 2-week at home bleaching protocol. There is no statistically difference between the two groups in $K_{IC}$ values.

**Fig 8.14:** Box and whisker plot visualizing *in situ* dentin $K_{IC}$ values in bleaching and placebo groups.
8.2 Analysis of Dentin Microhardness (KHN)

Values of hardness above 200 are considered extremes and were excluded from the analysis (3 in each group + 1 KHN data missing for a bleaching specimen). Independent samples t-test revealed no statistically significant difference in mean tooth hardness between bleaching ($M = 66.80$, $SD = 40.14$, $SE = 7.33$, $n = 30$) and placebo ($M = 53.35$, $SD = 42.52$, $SE = 8.04$, $n = 28$) groups, $t (56) = -1.239$, $p = .221$. The results for dentin hardness for the bleaching and the placebo groups along with their box plots are shown in Figures 8.15 and 8.16.

![Bar chart showing mean KHN for bleaching and placebo groups](chart.png)

**Fig 8.15:** Microhardness (KHN) of human dentin after *in situ* application of 10% carbamide peroxide and placebo gel, using 2-week at home bleaching protocol. No statistically significant difference in *in situ* dentin microhardness between the bleaching and the placebo groups was found.
Fig 8.16: Box and whisker plot visualizing *in situ* dentin microhardness (KHN) values in bleaching and placebo groups.

### 8.3 Reliability Analysis of Tooth Shades

Cronbach’s Alpha, calculated as the coefficient of reliability for three tooth shade measures determined by the investigator, the technician and spectrophotometer was ($\alpha = .829$, $N = 106$). The high value above 0.70 indicated as high degree of internal consistency for the three measures.

Pearson correlation coefficient and its significance were calculated for each of the three pairs of tooth shade measurements:

- Tooth shade determined by Investigator and Technician ($r = .704$, $n = 107$, $p < .001$)
- Tooth shade determined by Investigator and spectrophotometer ($r = .637$, $n = 114$, $p < .001$)
- Tooth shade determined by Technician and spectrophotometer ($r = .434$, $n = 112$, $p < .001$)

All three measures had positive significant association, with a strong relationship between Investigator and Technician, and between Investigator and spectrophotometer, and a moderate relationship between Technician and spectrophotometer.
8.4 Analysis of Changes in Tooth Shade

Mean and standard deviation were calculated for the bleaching and the placebo group for their baseline shades measured by Investigator, Technician and Spectrophotometer. There was no statistically significant difference between both groups in baseline shades. (Table 8.1)

<table>
<thead>
<tr>
<th>Baseline Tooth Shade</th>
<th>Bleaching group</th>
<th>Placebo group</th>
<th>Results of statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline tooth shades obtained by Investigator</td>
<td>14.16 (2.00)</td>
<td>14.04 (2.19)</td>
<td>$t(57) = -0.22, p = 0.83$</td>
</tr>
<tr>
<td>Baseline tooth shades obtained by Technician</td>
<td>15.59 (3.43)</td>
<td>15.00 (2.75)</td>
<td>$t(59) = -0.73, p = 0.47$</td>
</tr>
<tr>
<td>Baseline tooth shades obtained by Spectrophotometer (Vita Easyshade)</td>
<td>14.41 (2.12)</td>
<td>13.38 (2.47)</td>
<td>$t(61) = -1.79, p = 0.08$</td>
</tr>
</tbody>
</table>

Table 8.1: Baseline tooth shade measurements obtained by Investigator, Technician and Spectrophotometer, presented in shade tab value rank (higher shade value represents a lighter shade). There was no significant difference between both groups in baseline shades.

Before- and after- treatment tooth shades were compared. Using tooth shades measurements obtained by the Investigator, a significant improvement in tooth shades was determined in the bleaching group ($M = 1.70, SD = 1.86, SE = .36, n = 27, z = -3.757, p < .001$). There was no significant change in tooth shades in the placebo group ($M = .67, SD = 2.04, SE = .42, n = 24, z = -1.495, p = .135$). (Figures 8.17 and 8.20)
Fig 8.17: Box and whisker plot visualizing the change in tooth shade-tab value rank after 2-wk in situ treatment measured by Investigator.

Using tooth shades measurements obtained by the Technician, a significant improvement in tooth shade was determined in the bleaching group ($M = 1.89$, $SD = 3.65$, $SE = .69$, $n = 28$, $z = -2.541$, $p = .011$), while no significant change in tooth shades was determined in the placebo group ($M = 1.09$, $SD = 2.72$, $SE = .58$, $n = 22$, $z = -1.689$, $p = .091$). (Figures 8.18 and 8.20)

Fig 8.18: Box and whisker plot visualizing the change in tooth shade-tab value rank after 2-wk in situ treatment measured by Technician.
Using tooth shades measurements obtained by Spectrophotometer, a significant improvement in tooth shade was determined in the bleaching group \((M = 1.17, SD = 1.29, SE = .24, n = 30, z = -3.897, p < .001)\). No significant change in tooth shades was determined in the placebo group \((M = .25, SD = .85, SE = .17, n = 24, z = -1.633, p = .102)\). (Figures 8.19 and 8.20)

**Fig 8.19**: Box and whisker plot visualizing the change in tooth shade-tab value rank after 2-wk *in situ* treatment measured using Spectrophotometer (Vita Easyshade).

The before- and after- shade measurements by the Investigator, Technician and Spectrophotometer are summarized in Figure 8.20.

**Fig 8.20**: Mean change in tooth shade-tab value rank after 2-wk *in situ* treatment measured by Investigator (A), Technician(B) and the spectrophotometer(C).
<table>
<thead>
<tr>
<th>Change in tooth shade</th>
<th>Bleaching group</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (Standard Deviation, Standard Error)</td>
<td>N</td>
</tr>
<tr>
<td>Change in tooth shades obtained by Investigator</td>
<td>1.70* (1.86, 0.36)</td>
<td>27</td>
</tr>
<tr>
<td>Change in tooth shades obtained by Technician</td>
<td>1.89* (3.65, 0.69)</td>
<td>28</td>
</tr>
<tr>
<td>Change in tooth shades obtained by Spectrophotometer (Vita Easyshade)</td>
<td>1.17* (1.29, 0.24)</td>
<td>30</td>
</tr>
</tbody>
</table>

**Table 8.2:** Mean change in tooth shade-tab value rank after 2-wk *in situ* treatment in the bleaching and the placebo groups.

* Denotes statistically significant difference between bleaching and placebo group (p < 0.05).
8.5 Analysis of tooth sensitivity

A total of 4 treatment days were missed by the patients because of sensitivity or other reasons. The mean daily VAS scores over the course of treatment are graphically shown in Figure 8.21.

**Fig 8.21**: Mean visual analog tooth sensitivity scores for each day for bleaching and placebo group

Independent samples t-test revealed that participants in bleaching group ($M = 17.44$, $SD = 18.20$, $SE = 3.27$) had significantly higher mean visual analog score (VAS) than participants in placebo group ($M = 7.09$, $SD = 15.55$, $SE = 2.89$), $t (58) = -2.360$, $p = .022$ over the course of treatment. (Fig 8.22)

**Fig 8.22**: The mean VAS over course of treatment. There is a significantly higher mean VAS in the bleaching group.
Independent samples t-test revealed that participants in the placebo group ($M = 3.97$, $SD = 5.94$, $SE = 1.10$), $t (58) = -3.57$, $p = .001$ had a significantly fewer number of days with tooth sensitivity, compared to the bleaching group participants. ($M = 9.13$, $SD = 5.26$, $SE = 0.94$) (Fig 8.23)

**Fig 8.23:** Average number of days that participants experienced sensitivity for the bleaching and the placebo group.

A significantly greater proportion of the placebo group participants (65.5%) did not experience tooth sensitivity during the course of treatment, compared to the bleaching group participants (16.1%) ($\chi^2(2) = 15.228$, $p < .001$). (Fig 8-24)

**Fig 8.24:** The percentage of participants in the bleaching and the placebo group who did not experience tooth sensitivity during the course of treatment.
The sensitivity data for both groups were analysed to investigate the severity of the sensitivity that participants of both groups had experienced during the course of treatment. The sensitivity levels were determined using the VAS: Mild sensitivity (VAS: 1-33), Moderate sensitivity (VAS: 34-66), Severe sensitivity (VAS: 67-100). The graph shows the proportion of participants in bleaching group experiencing different levels of tooth sensitivity on a daily basis for the course of treatment. (Fig 8.25)

**Fig 8.25:** The proportion of participants in bleaching group experiencing different levels of tooth sensitivity on a daily basis for the course of treatment.

The graph below shows the proportion of participants in the placebo group experiencing different levels of tooth sensitivity on a daily basis in the course of treatment. (Fig 8.26)

**Fig 8.26:** The proportion of participants in the placebo group experiencing different levels of tooth sensitivity on a daily basis for the course of treatment.
The table below summarizes the instances (participant days) when various levels of sensitivity were reported (Table 8.3):

<table>
<thead>
<tr>
<th></th>
<th>Bleaching</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>No sensitivity</td>
<td>130 (32.0%)</td>
<td>281 (72.1%)</td>
</tr>
<tr>
<td>Mild sensitivity</td>
<td>207 (51.0%)</td>
<td>83 (21.3%)</td>
</tr>
<tr>
<td>Moderate sensitivity</td>
<td>45 (11.1%)</td>
<td>19 (4.9%)</td>
</tr>
<tr>
<td>Severe sensitivity</td>
<td>24 (5.9%)</td>
<td>7 (1.8%)</td>
</tr>
</tbody>
</table>

**Table 8.3:** The proportions of participants in both groups for the levels of sensitivity in the course of treatment.

These pie charts show the distribution of sensitivity levels between the bleaching and placebo groups for all participants and all days. Chi-square test confirms statistically significant difference in sensitivity between two groups, $\chi^2(3) = 128.11, p < .001$. (Fig 8.27)

**Fig 8.27:** The pie charts for displaying the distribution of sensitivity levels experienced by the participants in the bleaching and the placebo groups.
The tooth sensitivity statistics are summarized in Table 8.4.

<table>
<thead>
<tr>
<th>Sensitivity measure</th>
<th>Bleaching group (N = 31)</th>
<th>Placebo group (N = 29)</th>
<th>Results of statistical comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean daily sensitivity VAS score*</td>
<td>17.44 (18.20, 3.27)</td>
<td>7.09 (15.55, 2.89)</td>
<td>$t(58) = -2.36, p = .022$</td>
</tr>
<tr>
<td>Proportion of participants that have not experienced any tooth sensitivity*</td>
<td>16.1%</td>
<td>65.5%</td>
<td>$\chi^2(2) = 15.23, p &lt; .001$</td>
</tr>
<tr>
<td>Number of days experienced sensitivity*</td>
<td>9.13 (5.26, 0.94)</td>
<td>3.97 (5.94, 1.10)</td>
<td>$t(58) = -3.57, p = .001$</td>
</tr>
<tr>
<td>Change in sensitivity to $7^\circ C$ gel before and after bleaching</td>
<td>0.00 (0.62, 0.12)</td>
<td>0.04 (0.55, 0.11)</td>
<td>$bleaching: t(26) = .000, p = 1.000$, $placebo: t(23) = .371, p = .714$</td>
</tr>
<tr>
<td>Proportion of participants showing no change in tooth sensitivity to $7^\circ C$ gel before and after bleaching</td>
<td>63.0%</td>
<td>70.8%</td>
<td>$\chi^2(2) = .44, p = .804$</td>
</tr>
</tbody>
</table>

Table 8.4: Tooth sensitivity results for bleaching and placebo groups.
* Denotes statistically significant difference between bleaching and placebo group (p < 0.05)
8.6 Exploring Relationship Between Dentin Fracture Toughness, Microhardness, Tooth sensitivity and Shade Change

There was no statistically significant relationship among the following measured parameters: dentin fracture toughness, dentin microhardness, tooth sensitivity and shade change measured by Investigator, Technician and spectrophotometer- Vita Easyshade. (Table 8.5)

<table>
<thead>
<tr>
<th>Pair of variables</th>
<th>Placebo group</th>
<th>Bleaching group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dentin fracture toughness (KIC) and tooth hardness (KHN)</td>
<td>$r = .212$ ($p = .310$)</td>
<td>$r = -.045$ ($p = .832$)</td>
</tr>
<tr>
<td>Dentin fracture toughness (KIC) and mean VAS over the course of treatment</td>
<td>$r = -.184$ ($p = .350$)</td>
<td>$r = -.018$ ($p = .923$)</td>
</tr>
<tr>
<td>Dentin microhardness (KHN) and mean VAS over the course of treatment</td>
<td>$r = .121$ ($p = .572$)</td>
<td>$r = .276$ ($p = .192$)</td>
</tr>
<tr>
<td>Dentin fracture toughness (KIC) and shade change (Investigator)</td>
<td>$r = .101$ ($p = .637$)</td>
<td>$r = -.081$ ($p = .687$)</td>
</tr>
<tr>
<td>Dentin fracture toughness (KIC) and shade change (Technician)</td>
<td>$r = .105$ ($p = .642$)</td>
<td>$r = -.240$ ($p = .219$)</td>
</tr>
<tr>
<td>Dentin fracture toughness (KIC) and shade change Spectrophotometer (Vita Easyshade)</td>
<td>$r = .018$ ($p = .934$)</td>
<td>$r = -.095$ ($p = .619$)</td>
</tr>
<tr>
<td>Dentin microhardness (KHN) and shade change (Investigator)</td>
<td>$r = .185$ ($p = .436$)</td>
<td>$r = .279$ ($p = .221$)</td>
</tr>
<tr>
<td>Dentin microhardness (KHN) and shade change (Technician)</td>
<td>$r = -.128$ ($p = .601$)</td>
<td>$r = .165$ ($p = .453$)</td>
</tr>
<tr>
<td>Dentin microhardness (KHN) and shade change Spectrophotometer (Vita Easyshade)</td>
<td>$r = -.256$ ($p = .276$)</td>
<td>$r = -.177$ ($p = .408$)</td>
</tr>
</tbody>
</table>

Table 8.5: Correlation coefficients between pairs of parameters and statistical significance. No correlations were statistically significant.
8.7 Scanning Electron Microscopy Evaluation (SEM)

Representative SEM photomicrographs of the dentin fracture surface after 2-week treatment with bleaching and placebo gel are shown in Figure 8.28.

Generally, the SEM photomicrographs looked similar and no major differences were found between images of bleaching and placebo treated dentin fracture surfaces. In some of the SEM photomicrographs from the bleached dentin fracture surface, the lumens of the dentinal tubules were slightly widened in some areas, due to partial removal of peritubular dentin by demineralization in the tubule walls. The peritubular dentin often showed a few cracks along the long axis of a dentinal tubule. Cracks were rarely found in the placebo specimens.

![Image](image.png)

**Fig 8.28:** Scanning electron microscopy photomicrograph of dentin fracture surface after a 2-week treatment.

(A) Dentin fracture surface after 2-week placebo treatment.

(B) Dentin fracture surface after 2-week bleach treatment (10% carbamide peroxide).
The dentin fracture surface displayed the length of the dentinal tubules (T) surrounded by peritubular dentin (P) and intertubular dentin (I). The peritubular dentin is the slightly lighter region surrounding each tubule lumen, and intertubular dentin (I) is the slightly darker region which shows evidence of the collagen network as indicated by the slight variations in topography.
9. Discussion

This study is the first in situ study to evaluate the effects of bleaching on the mechanical properties of human dentin. It is also one of the very few studies that used $K_{IC}$ values to evaluate these effects. Unlike conventional strength test, $K_{IC}$ values do not change with different specimen geometries or loading conditions. In vitro, bleaching agents have been reported to decrease dentin fracture toughness and microhardness. However, fracture toughness tests have only been done in vitro which is not truly representative of clinical situations. Tooth bleaching treatments may not produce the same detrimental effects on dentin in situ. The in vitro studies need to be repeated in more clinically relevant situations where the important clinical factors affecting the results are considered.

Most of the previous studies examining the adverse effects of hydrogen and carbamide peroxide have focused primarily on enamel. Hydrogen peroxide diffuses through enamel to contact dentin indirectly and it may come into direct contact with dentin during the bleaching process. Testing the effect of bleaching materials on dentin is important since dentin forms the bulk of the tooth has a crucial role in supporting the brittle enamel and acts as stress absorbing cushion due to its organic matrix.

9.1 Dentin Fracture Toughness ($K_{IC}$) and Microhardness (KHN)

Few studies have measured $K_{IC}$ values for dentin. The values obtained for the fracture toughness and microhardness in our in situ study were comparable to those reported in earlier studies. The mean $K_{IC}$ for dentin in control and test groups ranged from 2.9 to 3.1 MPa.m$^{1/2}$ in different temperatures (El Mowafy & Watts, 1986) and 1 to 4.2 MPa.m$^{1/2}$ (L. E. Tam & Noroozi, 2007) and 1.4 to 3.5 MPa.m$^{1/2}$ (L. E. Tam et al., 2007) in other bleach studies. Imbeni, using fatigue-pre cracked three-point bend bar samples, reported $K_{IC}$ values in the range of 1.73 to 1.85MPa.m$^{1/2}$. (Imbeni et al., 2003) The mean $K_{IC}$ for the dentin specimens in the bleaching group and the placebo group in this in situ study was 2.22 and 2.26 MPam$^{1/2}$ respectively.
The compact test specimen is a standard configuration used for $K_{IC}$ testing. The specimen preparation aspects of this study were time consuming and required great care. According to ASTM E399, a state of plane strain is achieved when the sample thickness is $> 2.5 \left( \frac{Kc}{\sigma_y} \right)$ (where $\sigma_y$ = yield strength). This minimum thickness for dentin samples is estimated to be approximately 0.44 to 0.61 mm (El Mowafy & Watts, 1986) or 1.4mm (Imbeni et al., 2003) depending on the $\sigma_y$ value selected for use in the study calculations. (L. E. Tam et al., 2007) $K_{IC}$ values measured with similar specimens are indicative of fracture resistance because the minimum thickness criterion is generally quite conservative and because the plastic or damage zone of fracture is well contained within the specimen boundaries. (Imbeni et al., 2003; Nalla, Kinney, & Ritchie, 2003) The range of thicknesses for the specimens used in this study was between 1.50 and 2.38 mm.

Imbeni et al attributed the lower $K_{IC}$ values obtained in his study to a difference in the orientation of the dentinal tubules and to the effect of notch acuity on the $K_{IC}$ value. The fracture orientation of the dentinal tubules for the specimens in this study was “in-plane parallel” to the notch plane for all the specimens. This orientation was associated with a higher dentin $K_{IC}$ than a “perpendicular” orientation. (Iwamoto & Ruse, 2003; Nalla, Kinney, & Ritchie, 2003) The specimen preparation was done with great care to follow the standard configuration and keep the orientation of tubules the same in all of them. In our specimens, the fracture was initiated from the tip of the central notch which was sharpened with a fine blade and continued along the mid plane parallel to the dentinal tubules. The lack of a pre crack in the specimens may also have contributed to higher $K_{IC}$ values for this study compared with the results obtained in study done by Imbeni et al. (Imbeni et al., 2005) The use of a pre crack may be theoretically correct but is difficult to produce and measure. For composite resin materials, it was concluded that the use of a pre crack was somewhat impractical and that the use of sharply notched specimens provided precise data that are indicative of the $K_{IC}$ of the materials. (Ferracane et al., 1987)

In this study, dentin microhardness and surface analysis were also investigated after treatment with 10% carbamide peroxide using in situ methodology. The microhardness parameter is a surface property which can be indicative of abrasion resistance but not fracture resistance. It was presumed that the effects of direct bleach application would be more apparent on the surface of
dentin directly exposed to bleach than within the dentin. The average microhardness value (KHN) reported for dentin in the literature is in the range from 50 to 70 KHN, depending on the load applied (10-100 g). (Rodrígues JA. Basting RT. Serra MC. Rodrígues Júnior AL, 2001) The mean dentin microhardness for the bleaching and the placebo groups in our *in situ* study was 66.80 and 53.35 KHN, respectively. There was no statistically significant difference in microhardness between the bleaching and the placebo groups.

The results of the effects of the bleaching agents on the dentin mechanical properties can be affected by many different factors. The type of the dentin used in the experiment, the location in the tooth that the dentin specimen is prepared from; storage media, sterilization technique and dehydration of the specimen throughout the whole experiment are important factors.

Some of the values reported for dentin fracture toughness and microhardness are the results of the studies which used bovine or elephant dentin. Human dentin from extracted molars was used in this study. Since the largest source of variation in dentin comes from regional differences within a tooth, the location of the tooth from which the dentin specimen is taken and the orientation of the dentin tubules relative to the test load are important when measuring dentin mechanical properties. In our *in situ* study, only one sample was obtained from the same location from each tooth to minimize the effect of location on the results, as well as to maintain a consistent dentinal tubule orientation for fracture toughness testing.

The teeth used in this study were stored in 1% chloramine immediately after they were extracted. 1% chloramine is recommended as the storage media of choice since it has no significant adverse effect on dentin. (DeWald, 1997) Then the specimens were stored in artificial saliva and in 4°C. To sterilize the specimens, single dose gamma radiation (2.5MRad) was utilized. Gamma radiation is the preferred, safe and reliable sterilizing technique which does not alter the mechanical properties of dentin significantly. (Moscovich et al., 1999)

Keeping the specimens hydrated during the experiment was important since changes to the level of dentin hydration have been shown to affect dentin mechanical properties. (Jameson, Hood, & Tidmarsh, 1993)(Kinney, Marshall, & Marshall, 2003a; Kishen & Vedantam, 2007) It has been
reported that strain-at-fracture and fracture energy were significantly greater for hydrated and rehydrated dentin than for dehydrated dentin. (Jameson et al., 1993) A better simulation of clinical conditions is to store the specimens in an artificial saliva or calcium phosphate solution rather than in water or distilled water during the intervals between the daily bleach treatment times.

In this study, dentin specimens were placed in bleaching trays and were in contact with saliva intraorally during bleach treatment and were stored in artificial saliva when outside the oral cavity. Therefore, in our *in situ* study dentin dehydration was minimized and rehydration was maximized. Although some dentin dehydration might have occurred during the time of bleach application, it was expected that rehydration would occur by saliva during the treatment and at the time of artificial saliva storage. Both the bleaching and the placebo groups were subjected to the same potential dehydration and rehydration processes. Therefore differences in water content were not significant to cause significant differences in dentin fracture toughness and microhardness between the bleaching and the placebo groups.

Although enamel microhardness decreased and the surface morphology suffered a significant change after treatment with carbamide peroxide in a laboratory study, Smidt and others (1998) stated that the buffering capacity and the remineralization potential of saliva might overcome detrimental bleaching effects *in vivo*. When artificial saliva was used to simulate the natural saliva function, the detrimental effects of bleaching were less evident. (Rodrigues & others, 2001;de Freitas & others, 2002) In this study, dentin microhardness and surface analysis were also investigated after treatment with 10% carbamide peroxide using *in situ* methodology. The average microhardness value (KHN) reported for dentin in the literature is in the range from 50 to 70 KHN, depending on the load applied (10- 100 g). (Rodrígues JA. Basting RT. Serra MC. Rodrígues Júnior AL, 2001) The mean dentin microhardness for the bleaching and the placebo groups in our *in situ* study was 66.80 KHN and 53.35 KHN, respectively. There was no statistically significant difference in microhardness between the bleaching and the placebo groups.
Change in the Ca/P ratio of dental hard tissues after bleaching treatment has been reported, (Rotstein, Dankner, Goldman, Heling, Stabholz, & Zalkind, 1996) however, the amount was small and possibly clinically insignificant. (McCracken & Haywood, 1996) There was a correlation between the values of microhardness and calcium loss evaluations that was affected by the dynamics of the oral cavity. In a pH of less than 5.5, the amount of Ca and P in saliva was lower than the solubility rate of hydroxyapatite, with enamel having a tendency to lose Ca and P to the oral environment. (Cury, 1989) In the enamel slabs cycled in situ, saliva interfered in this process, allowing the reposition of mineral and the reestablishment of hardness values similar to nonbleached specimens. However, in enamel slabs cycled in vitro, there was no remineralization effect because of the absence of saliva. (Justino, Tames, & Demarco, 2004)

It has been shown that enamel (Ten Cate, 1990) and dentin (Rath, 1995) are more prone to remineralization when they have been demineralized. For example, deciduous enamel eroded by lemon juice was found to be more reactive than non-eroded enamel, and a higher fluoride deposition was observed in eroded enamel after applying 2% neutral sodium fluoride. When dentin is demineralized, ionic changes are induced, increasing mineral uptake, which replaces the mineral lost during treatment. (Rath, 1995)

Differences between the in situ and in vitro condition could be attributed to the important role of human saliva. Saliva has a buffering action due to the bicarbonate and phosphate systems. Some inorganic electrolytes contained in saliva (calcium, phosphorus and fluorides) are important participants in the remineralization process. When pH is under the physiologic limit, part of the calcium and phosphorus complexes are released and added to the ionic calcium and phosphorus reservoirs. (Thylstrup & Fejerskov, 1998) (Justino et al., 2004) So the remineralization effect of saliva could prevent the demineralization effect of bleaching treatment in human dentin in situ.

In the clinical situations the other important factor to reduce the adverse effects of the bleaching treatments on the dentin mechanical properties is the buffering capacity of the dentin itself. Dentin is highly effective as an acid buffer. Hydroxyapatite is a strong buffer for H+ ions; whole dentin is even more effective, perhaps because of additional effects of protein and/or other macromolecular components. Hydrogen ions from strong acids penetrate dentin poorly relative
to that from weak acids, or labelled water. The poor penetration is probably due to buffering of H
+ ions by hydroxyapatite and other components of dentin. Hydroxyl ions also diffuse less readily
than water. It is proposed that OH- ions also are buffered by displacing the less electronegative
phosphate ions from hydroxyapatite. (Wang & Hume, 1988) Also in in vivo, the pulp would
provide outward fluid pressure within the dentinal tubules. This could potentially reduce the
degree of bleach permeation into the dentin in the clinical situation compared with the in vitro
situation. Dentinal fluid in vital teeth could act as a buffer as well. (Camps & Pashley, 2000)
(L. E. Tam et al., 2007) It has been shown that the high buffering capacity of dentin and the high
reactivity of H+ insure that little H+ diffuses through dentin that is more than 0.6 mm thick.
(Camps & Pashley, 2000)

According to the manufacturer, the pH of the placebo gel and bleaching agent (10% carbamide
peroxide, Opalescence) was ~ 6.5. The data on strong acids indicates that buffering by
hydroxyapatite, can contribute to dentin’s protective effect in situations where the potential toxin
is strongly acid. There may be other mechanisms by which dentin protect the pulp against
chemical toxins from restorative materials. However, the three mechanisms that have been
described so far: diffusion limitation; limited wetness for hydrolysis; and buffering by dentinal
hydroxyapatite, appear to allow the relatively safe use of a wide range of tooth restorative
materials. (Hume, 1994)

A wide range of concentrations of the bleaching agents are available for use in studies and in
clinical products differs significantly. The concentration of bleach that was used in this study is
widely used in clinical practice. However, the use of higher bleach concentrations by dentists and
patients is growing. More severe changes in enamel microhardness and micromorphology have
been observed when higher bleach concentrations were used. (Shannon & others, 1993)
(Rodrigues & others, 2001). Although this study showed no significant difference in in situ
dentin fracture toughness and microhardness when 10% carbamide peroxide was used, the
reported dose-response effect of bleach on dentin fracture toughness in vitro (Tam et al) suggests
that a greater effect on dentin may be observed when higher bleach concentrations are used in
situ. (L. E. Tam et al., 2007) A subsequent in situ experiment using 15% carbamide peroxide is
currently underway.
Treatment times for the home bleaching technique also vary extensively in different studies and effects depend on how much time per day the patient spends applying the technique. (Cobankara, Unlu, Altinoz, & Fusun, 2004) More severe adverse effects have been reported in prolonged bleaching treatments. (L. E. Tam et al., 2007) In our study, bleaching agent and the placebo gel was applied for an average of 6.44 hours in 14 days using the at home bleaching technique, this application time is in agreement with that recommended by the manufacturer.

The application of bleach to the enamel, as opposed to the dentin, is the common intended clinical practice. When bleach treatment was applied directly to dentin, there was a significant reduction in dentin fracture toughness. (Tam, Kuo and Noroozi 2007) (Tam and Noroozi, 2007) When the bleach treatment was applied to dentin indirectly through intact enamel however, there was a smaller reduction in dentin fracture toughness in one study (Tam and Noroozi, 2007) and no significant differences in dentin KIC between the bleach and control groups in the other study (Tam, Kuo and Noroozi 2007). This suggested that, although it has been shown that peroxide penetrates through the enamel and dentin and forms measurable amounts of bleach within the tooth pulp, a variable thickness of the enamel and dentin could reduce the effects of carbamide peroxide and hydrogen peroxide on dentin mechanical properties. In our study we used the direct application method where bleach was applied directly to dentin. This has clinical relevance since bleaching materials may come to the direct contact with dentin during the bleaching treatment through occlusal attrition and cervical abrasions. This application method also enhanced the potential for salivary buffering capacity and surface remineralization effects on the dentin due to the direct contact of the dentin with saliva in the oral cavity, as opposed to dentin that is covered by enamel.

This in situ study is more clinically relevant than in vitro studies and the results should provide some reassurance that dentin is not overtly weakened by the bleaching protocol used in this study. However, the lack of a statistically significant difference can not be used to state that there is no effect of bleach on dentin fracture toughness or microhardness in situ. Further studies with a greater sample size or different study design are needed to find more evidence to accept or reject the null hypothesis.
9.2 Changes in Tooth Shade

Our objectives for monitoring the shade change were to confirm that a positive treatment effect was achieved and to investigate the correlation of the tooth shade results with fracture toughness and microhardness results.

Color classification, using a standard shade guide (for example, Vitapan Classical shade guide, Vita-Zahnfabrik, Bad Säckingen, Germany) with the buccal aspects of teeth, is the most frequent method used to compare the restored color with natural tooth color. (Matis et al., 2000; Meireles et al., 2008)

The shade evaluation was performed by experienced evaluators and the spectrophotometer under controlled clinical conditions. Considering the fact that there is also a relationship between subject age and the magnitude of whitening response (Gerlach & Zhou, 2001), in our study the average age for the bleaching and the placebo groups are 27.30 and 29.35 years respectively which are very similar. Reliability analysis also showed a high degree of internal consistency for all the shade measures.

Some studies have suggested that the color evaluation immediately after bleaching must be carried out with caution since tooth dehydration causes temporary color changes in teeth, especially when the bleaching gel is applied in combination with light exposure. It has also been suggested that because of alterations over time, color must be checked when water uptake is completed. (Rosenstiel, Gegauff, & Johnston, 1991) In our study the shade was measured 12-24 hours after the treatment was completed. It has been reported that the effective color change can be stable for up to two years. (Meireles, Santos, Bona, & Demarco, 2010)

In our study there was no statistically significant difference between the participants in the bleaching and the placebo groups in baseline shades. In this study, the significant improvement in the teeth shades that was observed in the bleaching group after data analysis using the tooth shades measurements obtained by the Investigator, Technician and Spectrophotometer,
confirmed the whitening effect on teeth by the bleach treatment. No significant change in tooth shades was determined in the placebo group. The results were consistent for the evaluators and the spectrophotometer. No statistically significant relationship was found between the shade change and any other measured parameters.

9.3 Tooth Sensitivity

Reports of sensitivity related to the use of traditional night guard vital bleaching agents are numerous. The percentage of participants reporting sensitivity has been reported to vary from 0 to 100%. (Reinhardt, Eivins, Swift, & Denehy, 1993; Sterrett, Price, & Bankey, 1995) More typical is the report by Haywood and others (Haywood et al., 1994) that, 52% of participants experienced tooth sensitivity and 31% experienced gingival sensitivity. A review of the literature estimated that sensitivity was a problem for two out of three people participating in clinical trials of night guard vital bleaching. (Haywood, 2000b) Tam evaluated 24 subjects using three different brands of 10% carbamide peroxide gel in a cross-mouth study; 64% of the subjects reported sensitivity in their daily log. (L. Tam, 1999)

While more studies report the incidence of sensitivity in clinical trials, some have reported severity. Schulte and others (Schulte, Morrissette, Gasior, & Czajewski, 1994) reported that sensitivity related to tooth whitening was severe enough to force participants to withdraw from their study. While 14% of participants discontinued bleaching due to sensitivity, 86% finished the study. Some of the high incidences of sensitivity reported in the literature are due to the fact that soft tissue sensitivity is included in the sensitivity category. (Browning et al., 2008) In our study, only tooth sensitivity was recorded and tissue sensitivity was avoided by giving detailed instructions regarding the amount of applied bleaching materials and by trimming and adjusting the custom tray margins carefully.

The validity and reliability of the VAS for measuring both experimental and clinical pain has been demonstrated by several investigators. Ekowski et al (1972), Joyce et al (1975), Ohnhaus & Adler (1975) have compared the VAS with other pain scales and the results indicate that the
VAS correlates well with these methods and appears to be more sensitive in discriminating between various treatments and changes in pain intensity. (Gillam & Newman, 1993) However, it has also been suggested that the VAS can only give a unidimensional assessment of pain, and as such cannot distinguish between the sensory, intensity and affective (unpleasantness) aspects of pain. (Gillam & Newman, 1993)

In our study 83.9% of the participants using the 10% carbamide peroxide gel and 34.5% of the participants using the placebo gel experienced some level of sensitivity. Most of the participants in both groups reported mild sensitivity and completed the course of the treatment. This is in agreement with previous reports indicating that home whitening treatment supervised by a dentist may result in sensitivity but that this side effect does not prevent the patient from successfully completing the full course of treatment. (Jorgensen & Carroll, 2002)

Jorgensen et al reported that the majority of the sensitivity reported by the subjects was mild and occurred with the carbamide peroxide gel, as well as with the inert placebo gel. (Jorgensen & Carroll, 2002') Mild tooth sensitivity can be expected in approximately 50% of patients who undergo home whitening treatment using the gel studied. Approximately 10% of patients may experience moderate sensitivity, and 4% of patients may experience severe sensitivity for one to two weeks. (Jorgensen & Carroll, 2002) In our study the proportions for mild, moderate and severe sensitivity were 51%, 11.1% and 5.9% respectively which is quite comparable to the proportions reported in the literature.

Our finding that sensitivity tends to decrease as treatment continues is also in agreement with findings in previous reports. (Mokhlis, Matis, Cochran, & Eckert, 2000)(Li, 1997)(L. Tam, 1999) There was no correlation between the tooth sensitivity and dentin fracture toughness or microhardness.

**9.4 Scanning Electron Microscopy**

One of the possible side effects of bleaching products is that the tooth structure may be weakened by oxidation of the organic or inorganic elements. (Seghi & Denry, 1992) Several
studies have evaluated the effects of these agents on tooth structure mainly using scanning electron microscopy. The results of these studies are contradictory. Haywood et al. (1990) reported no change in surface morphology with 10% carbamide peroxide. However, others have reported that carbamide peroxide caused minimal effect on the surface morphology of the enamel (R. H. Leonard Jr et al., 2001)(Bitter & Sanders, 1993)(Ernst et al., 1996)(Shannon et al., 1993) and local microstructural and chemical changes in dental hard tissues. (Rotstein, Dankner, Goldman, Heling, Stabholz, & Zalkind, 1996)(Cobankara et al., 2004).

Cobankara et al reported that there were no statistically significant differences between the surface roughness of untreated control specimens and the specimens treated with the bleaching materials (10% and 15% carbamide peroxide) for both enamel and dentin at any given measurement time. (Cobankara et al., 2004)(Kodaka et al., 1992).

Our SEM examination also did not provide sufficient evidence to conclude that there are significant differences in morphology between bleached and placebo treated specimen fracture surfaces. It has been shown that a controlled and continuous mineral formation facilitates remineralization and surface mechanical recovery of demineralized dentin. (Bertassoni et al., 2010)
10. Conclusions

It is important to note that the majority of the studies investigating adverse effects of bleach on tooth structure have evaluated these effects in vitro which is not representative of the in vivo and in situ situations. The similarities to the real clinical conditions provided by this in situ methodology can offer new perspectives in the evaluation of the effects of different bleaching materials and different techniques. Further investigations on bleaching are needed with a greater focus on clinical test methodologies, dentin (since there is much more literature on enamel than on dentin) and structural properties (rather than surface properties alone).

After dentin was embedded into a bleaching tray for an in situ 2-week at home bleaching treatment with 10% carbamide peroxide (n=34) and placebo gel (n=31):

1- No statistically significant difference in the dentin fracture toughness (K<sub>IC</sub>) values between the bleaching (M = 2.22, SD = .71) and the placebo (M = 2.26, SD = .86) groups was found (p = .818).

2- No statistically significant difference in the dentin microhardness (KHN) between the bleaching (M = 66.80, SD = 40.14) and the placebo (M = 53.35, SD = 42.52) groups was found (p = .221).

3- A statistically significant improvement in tooth shades was determined in the bleaching group using measurements obtained by the Investigator (M = 1.70, SD = 1.86, n = 27, p < .001), Technician (M = 1.89, SD = 3.65, n = 28, p = .011) and the spectrophotometer (M = 1.17, SD = 1.29, n = 30, p < .001). There was no significant change in tooth shades among the placebo group participants.

4- Participants in the bleaching group (M = 17.44, SD = 18.20) had significantly higher mean visual analog score (VAS) for tooth sensitivity than the placebo (M = 7.09, SD = 15.55) group (p = .022).
5-Participants in the placebo group (\(M = 3.97, SD = 5.94\)) had a significantly fewer number of days with tooth sensitivity, compared to the bleaching group participants (\(M = 9.13, SD = 5.26\)) (\(p = .001\)).

6-A significantly greater proportion of the placebo group participants (65.5\%) did not experience tooth sensitivity during the course of treatment, compared to the bleaching group participants (16.1\%) (\(\chi^2 (2) = 15.228, p < .001\)).

7-The bleaching group participants reported more often “moderate” and “severe” categories of tooth sensitivity than the participants in the placebo group.

8- No obvious differences were observed in dentin surface morphology between the bleaching and the placebo groups.
11. Study limitations

A major limitation of our results is the high standard deviations (SD) in both the fracture toughness tests (0.71 MPa.m$^{1/2}$ for the bleaching group and 0.86 MPa.m$^{1/2}$ for the placebo group) and the microhardness tests (40.14 KHN for the bleaching group 42.52 KHN for the placebo group).

These variations can be produced by factors such as heterogeneity in dentin, intraoral conditions, chemical composition of saliva, salivary flow, and variations in testing procedures that occurred during specimen preparation and loading. Some of the SD could have been reduced by paired testing for the microhardness tests by making an indentation on the same specimen before and after bleach treatment. This was not done in our study mainly because of infection control concerns. We wanted to minimize handling of the specimen after gamma irradiation.

Paired testing could also have been feasible for fracture toughness testing if left and right third molars from the same donor were used sequentially for placebo and bleach treatment by the same participant. This design will have its own limitation related to a safe washout period between placebo and bleaching treatments.

The fact that almost half of the participants were drawn from among students at the University of Toronto, Faculty of Dentistry might give rise to concern about possible bias because, as students, these participants were likely to be more interested in the study and, consequently, more motivated to use the at-home technique correctly than would participants from outside the school. On the other hand, having such a homogenous group of participants allowed us to make a more reliable comparison between the groups.

As previously discussed, the test did not reproduce the clinical situation because it provided direct access of the bleaching agent to dentin rather than indirectly through enamel. Regardless, there could have been the questionable uniform access of bleach to all regions of the dentin specimen because it was embedded in the bleaching tray and fracture toughness results may have been affected if there was variable penetration of bleach in the region of crack propagation.
Therefore it is possible that the bleaching agent did not reach the dentin specimen as much as it would in *in vitro* conditions. However, the hardness results would not have been affected because the polished surface of the dentin specimen that was subjected to hardness testing was placed facing the oral cavity and therefore directly received the bleach or placebo treatment. Placing the dentin specimen on the maxillary premolar tooth may have also exposed it to more salivary flow (due to proximity to the orifice of Stensen’s duct) and dilution of the bleaching agent.
12. Clinical Implications and Relevance

This study was designed to simulate the clinical procedures that are performed for dental bleaching as closely as possible. This study attempted to investigate the effects of 10% carbamide peroxide on dentin fracture toughness, microhardness, color change and sensitivity in situ. Changes to the structural integrity of dentin are of a great interest when bleaching is used as a treatment for whitening teeth. A small reduction in the fracture resistance of the tooth could have a great impact over the lifetime of the tooth as a result of fatigue and crack propagation. There are some in vitro concerns about the effects of bleaching agents on the mechanical properties of the dentin. The previously reported reductions in dentin fracture toughness and microhardness, however, were not confirmed in this in situ study. Yet we cannot conclude that there is no adverse effect on dentin as a result of the bleaching treatment with 10% carbamide peroxide from the results of this study (this would be at risk of committing a Type II error) because our findings of no significant difference could have been due to an inadequate sample size or study design.

We hope that data obtained as a result of this investigation could enhance the dental practitioners’ ability to more knowledgeably discuss the risks and benefits of home bleaching with their patients. Considering the greater relevancy of our in situ study to the clinical situations than in vitro studies, the reasonable sample size, and the overall similarity in the fracture toughness and microhardness numbers between the bleaching and placebo groups, we believe that dentists can be reassured by the results of this study to say that there is no obvious reduction in dentin structural integrity as a result of the bleaching protocol that was used in this study.

Clinically, it is quite common for a patient to repeat the bleaching procedure several times for several weeks or to use higher bleach concentrations in order to achieve a satisfactory lightening of tooth color. It is not known whether repeated bleaching or using a higher bleach concentration would cause a significant weakening of the dentin. The results of this study cannot be extrapolated to higher bleach concentrations or application times. Further studies are needed to be conducted to examine this.
13. References


Effect of tooth bleach on dentin structural integrity in situ.

Introduction

The researchers of this study include Dr. Dr. L. Tam (Associate Professor, Restorative Dentistry), Dr. H. Limeback (Associate Professor, Preventive Dentistry) and a specialty postgraduate student (Dr. P. Bahrami). They are affiliated with and can be contacted at the Faculty of Dentistry, University of Toronto, 124 Edward Street, Toronto, ON, M5G 1G6.

I understand that part of my curriculum at the Faculty of Dentistry includes an exercise in bleach tray fabrication and wear. For this reason, I have been asked to participate in a study, which will assess the *in situ* effect of carbamide tooth bleaches on dentin fracture toughness.

The purpose of this informed consent document is to provide information about the proposed study so that I may better make a decision as to whether I wish to participate. This consent form gives detailed information about the study and the possible risks and benefits. This proposed study will not change my studies at the Faculty of Dentistry. The student exercise in bleach tray fabrication and wear will proceed regardless of whether or not I agree to participate in the study.

Purpose of the Study:

The objective of this study will be to determine the effects of dental bleaches, applied in a conventional manner by patients, on the fracture toughness and hardness, or structural integrity, of dentin. The possibility of an association between changes in dentin fracture toughness and hardness, and changes in tooth color or tooth sensitivity additionally will be assessed.

If a decrease in *in situ* dentin fracture toughness or hardness is found, the results of this study will provide valuable confirmation to the *in vitro* literature that suggests that tooth weakening may occur as a result of direct bleach treatment. Tooth bleaching materials are available over-the-counter and patients may overuse these products in an attempt to further whiten their teeth. The findings of an *in situ* decrease in dentin fracture toughness that is related to the bleach application will suggest that the application of
bleach has the potential to decrease dentin structural integrity in vivo, and that bleach concentrations and times should be kept to a minimum.

Description of the Treatment and Study Method:
If I choose to participate in the study, the treatment and data collection procedures will consist of daily bleach or placebo at-home overnight applications for 14 nights using a bleach tray containing a sterilized dentin specimen that has been prepared from an extracted human molar, a color measurement, cold test and photograph taken before and after the bleach or placebo treatment.

Bleach or placebo treatment:
The bleach materials will include 10% or 16% carbamide peroxide (CP). Placebo gels, without the active CP, will be used as control materials. I will be free to choose which of the placebo or bleach materials I would want to use depending on the degree of tooth whitening I desire. Custom-made bleaching trays will be made for me to wear which will hold the bleach or placebo material as well as a sterilized dentin specimen. I will be asked to wear the bleaching tray, containing the dentin specimen, overnight for 14 nights. If I experience tooth sensitivity, I may choose to skip one or two days of bleaching. This is the typical method and treatment time used for tooth bleaching. The 14 nights of treatment should take place within a 28 day period. At the end of each daily bleach treatment, I will be asked to rinse the bleach tray containing the dentin specimen with tap water to remove all external traces of bleach and store the bleach tray with the dentin specimen in artificial saliva until the next bleach treatment. I will also be asked to record on a provided log form 1) the daily usage of the bleach or placebo tray wear, and 2) the degree of tooth sensitivity experienced on a visual analogue scale.

Data Collection:
The following procedures will be done for research purposes only and are not required as part of my usual bleach or placebo exercise:

A photograph will be taken of my front teeth only before and after treatment.
A cold test will be conducted before and after treatment. The cold testing will involve the placement of drop of cold (7°C) gel to the middle of one lateral incisor for 3 seconds. At the end of 3 seconds, the gel will be wiped off and I will be asked to score the lateral incisor tooth sensitivity on a visual analogue scale.
The color of my teeth will be assessed. A probe will contact the middle of one central incisor and a color reading will be taken.

Possible risks:
Tooth bleaching is a widely used treatment with no known long-term adverse effects. I may experience tooth sensitivity as a result of tooth bleaching and tooth sensitivity normally occurs in approximately 50% of patients who undergo at-home tooth bleaching. The tooth sensitivity is not known to persist beyond the immediate period of bleach treatment. I may also experience temporary gum irritation as a result of the tooth bleaching treatment. The additional procedures related to this study would not increase the degree of tooth sensitivity or gum irritation that I may experience. All dentin
specimens will have been sterilized prior to use and the risk of any disease transmission is not expected to be greater than that associated with routine dental treatment. There are no reasonably foreseeable risks or emotional distress associated with data collection procedures. I will need to spend extra time (15 minutes) to have the photographs, cold testing and color measurements taken.

Possible Benefits:
I will not benefit from participating in this study. Information from this study may help to determine the effects of tooth bleaches on dentin structural integrity.

Voluntary Participation:
If I choose to take part in this study, I do so of my own free will. I may refuse to take part now or can stop participating in this study at any time during the study period. If I do not wish to participate in any aspect of this study, my investigators will continue to treat me in the usual manner. My willingness or non-willingness to participate in the study will not affect my present or future academic performance, ie. participation will not grant me special academic consideration and refusal to participate will not lead to any academic repercussions.

Compensation/ Expenses:
I will receive $20 compensation for participation in this study. Partial compensation may be offered if I choose to withdraw during the study.

Confidentiality and Access to Medical and Dental Records:
At the conclusion of the research, the coded photographs and data obtained from the specimens will be kept on file by one of the investigators until publication of the research findings are complete or for five years, whichever comes first. Only the investigators will have access to the code. I will be informed in a timely manner if information becomes available that may be relevant to my willingness to continue participation in the study. I will not be informed of the results of the research. The researchers intend to publish the results of this research and if they do, my identity will remain confidential. Upon request to the investigators, I will have access to the list of scientific publications generated from this study following such publication.

Financial Support for the Study
This study is funded by a Faculty of Dentistry Research Fund.

Further Questions:
I have been given a copy of this consent form. After reading this, if I have questions, I should ask the investigator, or
Dr. D. Cvitkovich, Associate Dean, Institute of Dental Research, at 979-4765 X4592 who is not associated with this study, with whom I can discuss my rights as a research subject.

**TO BE SIGNED PRIOR TO REGISTRATION**

I have read and understand this consent form. My signature in this section of the consent form means that I agree to register into the study. I will keep a copy of this consent form.

________________________________________  ____________________________
Signature of Patient                        Date

________________________________________  ____________________________
Signature of Investigator                    Date

________________________________________  ____________________________
Signature of Witness                         Date
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Appendix-3 (Participants demographic, Specimens’ geometry, $K_{IC}$ and $KHN$ values)

**Bleaching group**

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| B-3       | 39.00 | D2 | B1 | 040 | 010 | B1 | B1 | 13 | 16 | 20 | 17 | 16 | 16 |
| B-4       | 63.00 | B2 | B1 | A1 | B2 | A1 | 14 | 16 | 15 | 14 | 15 |
| B-5       | 112.00 | B2 | B1 | A1 | 010 | B1 | B1 | 14 | 16 | 15 | 17 | 16 | 16 |
| B-6       | 110.00 | B1 | B1 | 040 | 020 | B1 | B1 | 16 | 16 | 20 | 18 | 16 | 16 |
| B-7       | 100.50 | A1 | B1 | B1 | 020 | A1 | B1 | 15 | 16 | 16 | 18 | 15 | 16 |
| B-8       | 84.00 | B1 | 020 | 010 | B1 | B1 | 16 | 18 | 17 | 16 |
| B-9       | 77.00 | A3 | B1 | A3 | 040 | A1 | B1 | 8 | 16 | 8 | 20 | 15 | 16 |
| B-10      | 77.50 | A1 | B1 | 030 | 020 | B1 | B1 | 15 | 16 | 19 | 18 | 16 | 16 |
| B-11      | 109.50 | A1 | B1 | B1 | 010 | D2 | B1 | 15 | 16 | 16 | 17 | 13 | 16 |
| B-12      | 73.00 | D2 | B1 | 030 | 010 | A1 | B1 | 13 | 16 | 19 | 17 | 15 | 16 |
| B-13      | 89.50 | D2 | B1 | A1 | 020 | A2 | B2 | 13 | 16 | 15 | 18 | 12 | 14 |
| B-14      | 111.50 | B1 | B1 | 030 | 020 | B1 | B1 | 16 | 16 | 19 | 18 | 16 | 16 |
| B-15      | 113.00 | B1 | B1 | 010 | 010 | B1 | B1 | 16 | 16 | 17 | 17 | 16 | 16 |
| B-16      | 112.00 | B1 | B1 | A1 | 030 | B1 | B1 | 16 | 15 | 16 | 19 | 16 | 16 |
| B-17      | 50.50 | B1 | B1 | 020 | 020 | A1 | B1 | 16 | 16 | 18 | 18 | 15 | 16 |
| B-18      | 96.00 | D2 | A1 | A2 | A1 | B2 | B1 | 13 | 15 | 12 | 15 | 14 | 16 |
| B-19      | 109.00 | D2 | B1 | D2 | 030 | D2 | B1 | 13 | 16 | 13 | 19 | 13 | 16 |
| B-20      | 58.00 | C2 | C2 | D3 | D2 | A3.5 | C2 | 10 | 10 | 7 | 13 | 5 | 10 |
| B-21      | 101.00 | B2 | B1 | A1 | 040 | A1 | B1 | 14 | 16 | 15 | 20 | 15 | 16 |
| B-22      | 73.50 | B1 | B1 | 040 | 040 | B1 | B1 | 16 | 16 | 20 | 20 | 16 | 16 |
| B-23      | 45.00 | D2 | D2 | B2 | B2 | 13 | 13 | 14 | 14 |
| B-24      | 99.50 | C1 | B1 | C1 | B1 | C1 | D2 | 11 | 16 | 11 | 16 | 11 | 13 |
| B-25      | 38.00 | B1 | 010 | 010 | B1 | B1 | 16 | 17 | 17 | 16 | 16 |
| B-26      | 37.00 | B1 | B1 | 030 | 010 | B1 | B1 | 16 | 16 | 19 | 17 | 16 | 16 |
| B-27      | 68.50 | B1 | B1 | 040 | 030 | A1 | B1 | 16 | 16 | 20 | 19 | 15 | 16 |
| B-28      | 103.00 | A1 | B1 | B1 | 010 | B1 | B1 | 15 | 16 | 16 | 16 | 15 | 16 |
| B-29      | 88.50 | A2 | A1 | A1 | B2 | A1 | 12 | 15 | 15 | 14 | 15 |
| B-30      | 92.50 | A1 | B1 | B1 | 030 | B2 | B1 | 15 | 16 | 19 | 14 | 16 | 16 |
| B-31      | 105.50 | A1 | B1 | 040 | B2 | B1 | 15 | 16 | 20 | 14 | 16 |
| B-32      | 89.50 | B2 | B1 | D2 | A1 | B1 | 14 | 16 | 13 | 15 | 16 |
| B-33      | 87.50 | B1 | B1 | A1 | 16 | 16 | 15 | 15 |

V1: Investigator  
V2: Technician  
EZ: Spectrophotometer (Vita Easyshade)
Placebo group:

| Participant | AGE | Group | Placebo | HRS | PreShade-V1 | PostShade-V1 | PreShade-V2 | PostShade-V2 | PreShade-EZ | PostShade-EZ | PreShade-V1 | PostShade-V1 | PreShade-V2 | PostShade-V2 | PreShade-EZ | PostShade-EZ |
|-------------|-----|-------|---------|-----|-------------|--------------|-------------|--------------|-------------|--------------|-------------|--------------|-------------|--------------|-------------|--------------|--------------|
| P-1         | 24  | B1    | B1      | 040 | 030         | B1           | 010         | B1           | B1          | 16           | 16          | 20           | 17          | 16          | 16          | 16          |
| P-2         | 24  | C2    | B1      | 030 | B1          | C1           | 010         | B1           | 16           | 16           | 19          | 16          | 11          | 11          | 11          | 11          |
| P-3         | 21  | D2    | D2      | 040 | A1          | B1           | A1          | B2           | 16           | 16           | 13          | 13          | 13          | 13          | 16          | 12          |
| P-5         | 29  | A2    | D2      | 040 | B2          | A1           | B2          | B1           | 16           | 16           | 13          | 16          | 14          | 14          | 14          | 14          |
| P-6         | 22  | B1    | B1      | 040 | B1          | A1           | B2          | B1           | 16           | 16           | 13          | 20          | 14          | 15          | 15          | 15          |
| P-7         | 26  | A1    | B2      | 020 | B2          | A1           | B2          | B2           | 15           | 15           | 18          | 13          | 13          | 13          | 13          | 13          |
| P-8         | 27  | C1    | B1      | 040 | B2          | A1           | B2          | B1           | 11           | 16           | 13          | 13          | 12          | 16          | 13          | 13          |
| P-9         | 23  | B1    | B1      | 030 | A1          | A1           | A1          | A1           | 16           | 16           | 15          | 19          | 15          | 15          | 15          | 15          |
| P-10        | 33  | A1    | B2      | 030 | A1          | A1           | A1          | A1           | 15           | 14           | 16          | 15          | 15          | 15          | 15          | 15          |
| P-13        | 21  | B1    | B1      | 030 | B1          | B1           | A1          | B2           | 16           | 16           | 15          | 20          | 16          | 16          | 16          | 16          |
| P-14        | 22  | B1    | B1      | 040 | B1          | B1           | B1          | A1           | 16           | 16           | 15          | 16          | 14          | 14          | 14          | 14          |
| P-15        | 25  | A1    | A1      | 030 | A1          | A1           | A1          | A1           | 15           | 14           | 16          | 15          | 15          | 15          | 15          | 15          |
| P-17        | 50  | A1    | A2      | 020 | A1          | B2           | B2           | A1           | 15           | 15           | 15          | 15          | 15          | 15          | 15          | 15          |
| P-19        | 45  | A1    | A2      | 020 | A1          | A1           | B2           | B1           | 12           | 13           | 16          | 15          | 15          | 15          | 15          | 15          |
| P-20        | 31  | A1    | A1      | 020 | A1          | A1           | A1          | A1           | 16           | 16           | 19          | 20          | 13          | 13          | 13          | 13          |
| P-21        | 24  | A1    | A1      | 020 | A1          | A1           | A1          | A1           | 15           | 15           | 15          | 15          | 15          | 15          | 15          | 15          |
| P-22        | 27  | A1    | A1      | 020 | A1          | A1           | A1          | A1           | 15           | 15           | 15          | 15          | 15          | 15          | 15          | 15          |
| P-23        | 30  | A1    | A1      | 020 | A1          | A1           | A1          | A1           | 15           | 15           | 15          | 15          | 15          | 15          | 15          | 15          |
| P-24        | 33  | A1    | A1      | 020 | A1          | A1           | A1          | A1           | 15           | 15           | 15          | 15          | 15          | 15          | 15          | 15          |
| P-25        | 31  | A1    | A1      | 020 | A1          | A1           | A1          | A1           | 16           | 18           | 16          | 15          | 16          | 16          | 16          | 16          |
| P-26        | 55  | A1    | A1      | 020 | A1          | A1           | A1          | A1           | 16           | 18           | 16          | 16          | 16          | 16          | 16          | 16          |
| P-27        | 29  | B1    | B1      | 030 | A1          | B1           | B1           | B1           | 16           | 18           | 16          | 16          | 16          | 16          | 16          | 16          |

V1: Investigator
V2: Technician
EZ: Spectrophotometer (Vita Easyshade)
## Appendix-5 (VAS-Sensitivity data)

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**BL-S**: Base Line Sensitivity

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V1: Investigator
V2: Technician
EZ: Spectrophotometer (Vita Easyshade)