THE EFFECTS OF DENTINE CONTAMINATION ON THE SHEAR BOND STRENGTH OF A SELF-ETCHING ADHESIVE AND A NANOCOMPOSITE.

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This research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science in Dentistry.

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I, Dr Vishani Soni declare this research report is my own work. It is being submitted as a partial fulfilment for the Masters of Science degree in Dentistry by coursework and research report at the University of Witwatersrand, Johannesburg. This research has not been previously submitted before any other degree or examination.

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ABSTRACT

**Purpose:** Resin restorative materials have improved over the years. A major obstacle to the acquisition of acceptable bond strength of bonding agents is the presence of contaminated dentine cavity preparations. The purpose of this study was to investigate the effects of oral contaminants such as blood, saliva and a disinfectant contamination on the shear bond strength of a nanocomposite on a self-etching adhesive system.

**Materials and Methods:** Thirty-six caries free premolar teeth were extracted and placed in a disinfectant solution containing 0.5 % Chloramine T solution, and then randomly distributed into four groups. Each tooth was then placed into a stainless steel ring supported by clear self-curing acrylic. They were thereafter immersed into a saline solution of 37 °C - 37.5°C in an incubator for 24 hours. The enamel surfaces of the premolars were then ground with a Pro-trim 1725 Hertz grinder using 600 grit silicon carbide fine grinding paper to expose the dentine surface of each tooth. The sample was then re-immersed in the saline solution and incubated at 37°C - 37.5°C. The teeth were then arranged into the four groups: Group 1 (control group); Group 2 (human blood contamination at 5 seconds); Group 3 (human saliva contamination at 5 seconds) and Group 4 (chlorine dioxide contamination at 5 seconds). A self-etching adhesive bonding system (Scotchbond universal™) and Filtek supreme XTE composite was applied to the exposed dentine surface. Samples were randomised and then sheared using an Instron testing machine to determine their bond strengths. The fractured components of each sample were measured, compared and further examined under a stereo microscope to determine the modes of failure. The data were analysed using a one-way analysis of variance (ANOVA) and the level of significance was set at a p-value of less than 0.05.
Results: A significant difference was found in the shear bond strength between the control (group 1) and the blood contaminated group (group 2) (p-value of 0.00064). The chlorine dioxide group (group 4) that had no effect on shear bond strength to dentine (p-value of 0.55). Adhesive failures (between bonding agent and dentine) were predominant in group 2 and to a lesser extent in group 3. Most group 4 samples had cohesive fractures (within the dentine).

Conclusion: The bond strength to dentine using a self-etching adhesive was reduced when contaminated with blood. Group 2 samples (blood) caused significantly greater bonding failure as compared to all the other groups. Chlorine dioxide solution is a powerful disinfectant and does not affect the bonding to dentine. The null hypothesis statement, which stated that there was no difference in the shear strength between any of the conditions, was thus rejected. Further studies on the application of chlorine dioxide as a disinfectant on cavity preparations need to be considered given the surprising positive results of chlorine dioxide group.
ACKNOWLEDGEMENTS

The author would like to convey her sincerest gratitude and appreciation to the individuals who have assisted and guided her in completing this research.

Firstly, this would not be possible without the Almighty who gave me the strength to complete what I had started.

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LIST OF ABBREVIATIONS

Bis-GMA: Bisphenol-A-glycidyl dimethacrylate
TEDGMA: Triethylene glycol dimethacrylate
UDMA: Urethane dimethacrylate
HEMA: Hydroxyethyl methacrylate
Bis-EMA: Ethoxylated bisphenol-A-glycol dimethacrylate
MMP: Matrix metalloproteinases
WHO: World Health Organisation
FDI: World Dental Federation
μm: Micrometres
nm: Nanometres
CHAPTER 1:

INTRODUCTION AND LITERATURE REVIEW

1.1. Resin composites

1.1.1 Brief History

Resin composite filling materials are a modern class of aesthetic dental restorative materials that comprise of inorganic fillers within a resinous matrix (Zimmerli et al., 2010). It was the initial discovery of bisphenol-A-glycidyldimethacrylate (Bis-GMA), with its ability to bond with these fillers, that paved the way for the first composite resin dental restorative material, Bowen’s resin (Bowen, 1956; Durner et al., 2012; Pitel, 2013). Bowen’s resin was studied and improved by Buonocore (1962), who first described the ability to bond this composite to tooth structure by acid etching with phosphoric acid (Swift, 2002).

The early resin composites were recommended for use on anterior dentition (Chan et al., 2010). It was their low compressive strength resulted in their high rate of occlusal wear that made them unsuitable for use as a posterior restoration (Hendriks et al., 1986; Dejak et al., 2015). Occlusal wear was reduced by the addition of glass fillers to the resins which became alternative materials to amalgam fillings as posterior restorations (Hendriks et al., 1986; Dejak et al., 2015). Resin composite materials provide some clinical advantages over amalgam restorations such as their ability to bond to enamel, to provide micro-mechanical adhesion to tooth structure and to increase the strength of the tooth structure with minimum
stress and fracture (Chan et al., 2010; Pongprueksa et al. 2016). Amalgam fillings have retention failure as a result of their inability to chemically bond to the tooth structure and the need for more extensive preparation of healthy structures that may be required for material retention, which can in turn make the teeth more prone to fracture (Firouzmandi et al., 2016).

The replacement of amalgam by composite restorations was encouraged by the WHO (World Health Organisation) and the FDI (World Dental Federation) who have both stated that the high mercury content of amalgam is still considered toxic to the patient and the dentist. Their reports showed that the mercury toxicity had developed due to these two major dental problems: the clinical negligence in the effective removal of amalgam waste and the amount of mercury indoor vapour being generated in the dental workspace environment upon removal of old amalgam fillings. These problems could lead to amalgam particles being absorbed into patients’ oral cavity and mercury vapour being inhaled by the dentist and the patient, which may cause severe health problems (Ritchie et al., 2004). Environmental and water pollution by the improper disposal of the mercury particle must also not be overlooked. Therefore many first world countries have restricted the use of amalgam as a filling material. However, the routine replacement of amalgam by resin composite filling materials is controversial and debated by some clinicians (Chan et al., 2010).

Resin composites consists of three major phases that produces a more favourable restorative material (Lutz et al., 1983; Peutzfeldt, 1997): 1) Organic phase (the matrix), 2) Interface phase (coupling agent), and Dispersed phase (the fillers).

The organic phase consists of monomers, an initiator for free radical polymerisation and a stabilizer to chemically stabilise the cured resin composite. The interface phase consists of the organo-silane that chemically bonds the filler to the resin matrix. The dispersed phase with inorganic fillers consists of glass, quartz or silica. The precise relationship between
fillers and their mechanical properties would enable one to establish a composition for the material to perform optimally under clinical conditions (Braem et al., 1989).

1.1.2: Filler materials

Fillers are made of quartz, ceramic or silica (Zimmerli et al., 2010). Inorganic fillers particle were measured and reported to reduce polymerisation shrinkage when converted into a solid filler by the addition of covalent bonds, which reduced the interatomic distance (Pitel, 2013). Solid filler particles were added to reduce the total amount of monomer used, to help maintain constant volumetric dimensions during the polymerisation shrinkage of monomers. This provides strength, optimal aesthetic properties and improves on the clinical performance of resin composites (Pitel, 2013). Therefore, as filler density increases, polymerisation shrinkage is reduced because shrinkage is confined to the monomer phase of resin composite. Total filler load is also dependant on the packing property of the solid fillers which depends on the shape and size of these filler particles. Polishing of any composite material is also dependent on the type, morphology and particle size of the fillers (Lin et al., 2013; Tabatabaei et al., 2013). Below is a summary of composite materials that have been subdivided into their compositional filler sizes, materials and their interactive matrixes that developed over time (table 1.1).
### Table 1.1 Resin Composites and Filler materials

<table>
<thead>
<tr>
<th>Composite type</th>
<th>Filler size</th>
<th>Filler Material</th>
<th>Resin + Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrofilled</td>
<td>10-40µm</td>
<td>Quartz or barium</td>
<td>Bis-GMA / TEGDMA</td>
</tr>
<tr>
<td>(1970’s)</td>
<td></td>
<td>glass</td>
<td></td>
</tr>
<tr>
<td>Microfilled</td>
<td>0.01-0.1µm</td>
<td>Colloidal silica</td>
<td>Bis-GMA and TEGDMA, UDMA</td>
</tr>
<tr>
<td>(1970’s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hybrid (1980’s)</td>
<td>15-20µm and</td>
<td>Glass and colloidal</td>
<td>Bis-GMA, UDMA, HEMA</td>
</tr>
<tr>
<td></td>
<td>0.01-0.05µm</td>
<td>silica</td>
<td></td>
</tr>
<tr>
<td>Modern Hybrid (1990’s)</td>
<td>0.05-0.1µm</td>
<td>Glass, Zirconia and</td>
<td>Bis-GMA / TEGDMA ; UDMA,</td>
</tr>
<tr>
<td></td>
<td>0.01-0.05µm</td>
<td>/colloidal silica</td>
<td>Bis-EMA (resin)</td>
</tr>
<tr>
<td>Nanofilled (2000’s)</td>
<td>10nm;</td>
<td>Silica or zirconia;</td>
<td>Bis-GMA, TEGDMA, UDMA,</td>
</tr>
<tr>
<td>and</td>
<td>20nm (silica)</td>
<td>and silica and</td>
<td>Bis-EMA (resin)</td>
</tr>
<tr>
<td>Nanocomposites (2005’s)</td>
<td>4-11nm (zarconia)</td>
<td>zarconia</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations:
- Bis-GMA: bisphenol-A-glycidyldimethacrylate
- TEDGMA: triethylene glycol dimethacrylate,
- UDMA: urethane dimethacrylate,
- Bis-EMA: ethoxylated bisphenol-A- glycol dimethacrylate,
- µm-micrometres
- Nm-nanometres

#### 1.1.1.2 Composite types (based on changes in filler particles)
Macrofilled composites developed in the 70s, have large, more rounded inorganic particles within a smaller spaced matrix that have the advantage of increased compressive strength and
were generally considered for restoration of the posterior dentition (Manhart et al., 2000; Chan et al., 2010). However, these composites had the major disadvantages of poor wear resistance and poor lustre when polished (Lutz et al., 1983; Manhart et al., 2000). These were then replaced by the smaller sized radiolucent inorganic glass spheres within a larger matrix field called microfilled composites in the late 70s (table 1.1), to improve on the polishability of the resin.

However, microfilled composites had a high filler content makes them extremely viscous, difficult to manipulate and adapt to prepared occlusal surfaces resulting in further adhesive failures (Lutz et al., 1983). Furthermore, a high incidence of secondary decay was commonly observed under fractured composite restorations. Increasing the filler content and reducing the filler size were methods to improve on the strength and wear resistance of resin composites on posterior dentition (Manhart et al., 2000). Both macrofilled and microfilled composite materials have variations in their filler sizes. The viscosities of these composites made the clinical handling of these materials a challenge to some dentist (Chan et al., 2010). These composites were then enhanced by diluting their monomers with a low viscosity monomer, triethylene glycol dimethacrylate (TEDGMA) and urethane dimethacrylate (UDMA), leading to the development of the hybrid composite materials.

Hybrid composite materials are defined as the combination of inorganic macrofillers, microfillers and pyrogenic organic silica that were added to the original matrix to improve on the viscosity and wear resistance of the resin composite material (Lutz et al., 1983). Hybrid composites consist of bisphenol-A-glycol dimethacrylate (Bis-GMA), TEGDMA, UDMA (e.g. Filtek 250 and Z100 composite materials). These composites have a combination of large fillers and colloidal silica or submicron fillers. Their initial development of the 0.01 μm
filler particles were the smallest filler particles ever developed. Hybrid composites unfortunately had poor aesthetic properties (Lutz et al., 1983) and extensive shrinkage and translucency after polishing was a result of their different filler types and volume (50-60%) (Lutz et al., 1983; Ikejima at al., 2003)

Additionally, there were changes to the resin matrix by incorporating more structures such as UDMA. This resulted in a substantial improvement in their mechanical properties and adhesion of the resin composite to the tooth structure (Asghar et al., 2010; Akimoto et al., 2011). Improvements included a range of factors, such as: reduction of the inherent weakness of the material, reduction in microleakage (Tay et al., 2001), enhanced aesthetics and improved wear resistance against high occlusal forces (Ausiello et al., 2001).

Further research led to the development of the nanocomposites in the 21st century (table 1) (Mitra et al., 2003; Flury et al., 2014). These nanofiller particles form nano-clusters designed to improve the strength of direct restorative materials (table 1.1). They differ from other composites by their filler sizes and materials (table 1.1). There are different types of nanocomposites containing the nanofiller particles in capsules namely Filtek Supreme XTE Universal restoration, Gardia Forte (Japan) and Lune-Wing (Japan). Different nanocomposite have different compositions. Filtek Supreme XTE Universal restoration differs from Gardia Forte and Lune-wing by its composition of Bis-GMA, UDMA, TEGDMA and ethoxylated bisphenol-A- glycol dimethacrylate (bis-EMA resins (Choudhary et al., 2013; Leal et al., 2015). This nanocomposite is a polymer based, multiphase solid material providing an increase in the quantity of nano-filler particles (78, 5%) to resin matrix (Lin et al., 2013). This enhances their mechanical and physical properties, as well as their adhesion to tooth structure (Rueggeberg et al., 1990; Gupta et al., 2015; Besinis et al., 2015). Filtek Supreme XTE comprises of four opacities which follow in the order of application namely dentine (most
opaque), body, enamel and then translucency. These opacities provides a better tooth shade. This nanocomposites has improved wear resistance (Mitra et al., 2003), reduced shrinkage (Leal et al., 2015) and improved the structural bond with dentine (Li et al., 2014). Their structure provides superior polishability, enhanced aesthetics and improves on translucency. There have been attempts to improve on their antimicrobial properties by the addition of fluoride ions (re-mineralizes tooth structures) and silver ions (protective function against S.Mutan infections). However, these ions had an adverse effects on the mechanical properties and colour of the restorative materials (Zimmerli et al., 2010). If correctly used by application in layers, a dry operating field and sufficient polymerisation, it can be a reliable restorative material for all application (Zimmerli et al., 2010).

It can be used for core build up, splitting, and indirect restorations and is considered to be the preferred material for direct anterior and posterior restorative applications (Mitra et al., 2003; Palaniappan et al., 2009, Jin et al., 2014).

1.2 Adhesion

Adhesion as a dental term is used to define the ability of a material to bond to tooth structure and to other materials. The basic principle of adhesion is that the bond must be strong, durable and should adhere to both enamel and dentine. Adhesion of composite resin to the tooth structure allows for the planning of more conservative cavity preparation. These cavity wall preparations are usually rounded and/or curved as compared to obtuse / ninety degree angles for the amalgam filling retention.

Composite materials can “reinforce the remaining tooth structure by better distributing the functional stresses across the bonding interface” (Firouzmandi et al., 2016). A high rate of mechanical failure of resin composite materials was caused by polymerization shrinkage at the interface between the restoration and the tooth tissues (Peutzfeldt, 1997). The viscoelastic
properties of resin composites play a vital role in maintaining bonding to cavity wall preparations and are dependent on two important factors (Braga et al., 2005):

1. The confinement of the material against the cavity wall depends on the percentage of composite surface that is bonded to the substrate in relation to the total tooth surface area.

2. The compliance of the bonding substrate depends on the type of bonding agent used and the tooth histology.

The ideal bonding method between a tooth surface and restoration is difficult to achieve due to a number of reasons, including: the composition of the dentine, the presence of contamination and moisture. To better understand adhesion, it can be subdivided into two major types: Chemical and micromechanical adhesion.

1.2.1 Chemical versus micro-mechanical adhesion

Chemical adhesion is defined as the absorption of molecules that penetrate the tooth substrates by rearranging atoms to form covalent or ionic bonds (primary forces) or hydrogen bonds /van der Waal forces (secondary forces) (e.g. Glass ionomer materials).

Micro-mechanical adhesion is the ability of materials to flow into the surface irregularities of tooth surfaces and set under a light source, so forming a micro-mechanical lock with tooth structures.

Chemical and micromechanical adhesion to tooth structure is complicated by the complex nature of the tooth’s micro-anatomy which requires an understanding of the organic and inorganic tooth structures of enamel and dentine.
1.2.1.1 Bonding to enamel

Enamel consists of 95% hydroxyapatite crystals packed in prisms, 1-4% of enamelin and 3% water. The prisms constitute the main fraction from the outermost surface of the enamel to approximately 5 microns from the dentoenamel junction. They have inorganic enamel rods that follow specific patterns within the tooth structure. These rods generally run vertically on the occlusal surface and horizontally mesially and distally to the tooth structure and can cross over each other. The enamel surface varies in thickness from 2.5mm from the cusp tapering to the cementoenamel junctions (CEJ).

Enamel can be weakened by bacteria that cause demineralisation which may extend into the dentine and pulpal regions of the tooth ultimately causing toothache (Jia et al., 2013). Removal of the decay and preparation of the cavity for the placement of a bonded filling requires the use of phosphoric acidic etchant on the enamel structures. Initially concentrations of 85% phosphoric acidic etchants were used to etch human enamel (Swift, 1998). These concentrations have been discontinued due to their deleterious effects on pulpal tissue, which devitalised healthy teeth. Current recommended concentrations of 34-37% phosphoric acid are used because this changes the enamel tomography from a low reactive surface to one that is more susceptible to adhesion with a minimal demineralization of the prismatic enamel (Zidan et al., 1986; Lopes et al., 2007). The different angulation of the prism crystals causes the acid to demineralize certain micro-regions on the enamel surfaces (Lopes et al., 2007). The type of acid and application time has to also be considered (Whittaker et al., 1982). An acid concentration of 35% was recommended to etch tooth structures for 20-30 seconds.
Etching the enamel creates a rough cobblestone surface with porosities exposing the enamel rods to the micro-mechanical adhesion of the resin materials by the formation of resin tags (fig 1). In addition, adhesion to enamel may be influenced by the specific structure of the prismatic enamel and interprismatic enamel (Nanci, 2013). These specific structures reinforce the enamels’ anatomical thickness, which has additional benefits to adhesion. Bevelling the cavo-surface enamel margin will also increases the enamel area to the adhesion substrate-resin monomer application (Coelho-de-Souza et al., 2010).

In the earlier phases of adhesive dentistry, bonding to enamel was stronger than bonding to dentine (Van Meerbeek et al., 1994; Manuja et al., 2011) because of the inorganic structure of enamel and the regular arrangement of its hydroxyapatite crystals. Bonding to dentine had to be improved.
1.2.1.2 Bonding to Dentine

Dentine has a unique organic structure that consists of 70% hydroxyapatite crystals, 10-20% organic matrix and 10% water. They are 1.6mm to 1.8mm thick (Perinka et al., 1992) containing dentinal tubules that are surrounded by a hypermineralized layer (peri-tubular dentine) and intertubular matrix that consists of type 1 collagen, non-collagenous proteins and proteoglycans (Tjäderhane et al., 2013). The tubules are reinforced by apatite crystals which partition the collagen network into extrafibrillar, and intrafibrillar mineral. They prevent the collagen framework from collapse. The mineralization of dentine requires an adequate supply of water particles to surround the fibrils in order to transport nutrients to the fibrils. Once they are fully mineralized dentine becomes impenetrable to dental materials. The collagen network that surrounds the dentinal tubules contains mineralized cells such as odontoblasts and odontoclasts facilitating remodelling and re-mineralization of the dentine. Odontoblast cell lay down new dentine while odontoclast remove dentine fragments facilitating dentine mineralization and growth.

While the dentine is thicker than enamel, it is readily penetrated by biological and chemically aggressive microorganisms within the oral fluids (John et al., 2015) due to certain enzymes produced by the dentine such as matrix metalloproteinases (MMP) and cathepsin. These enzymes and the host enzymes breakdown the collagen framework (Pashley et al., 2011) and destroy the bonding surface (Tjäderhane et al., 2013).

Dentine bonding is therefore more difficult and less predictable than bonding to enamel (Swift, 2002). Studies have shown that failure of dentine bonding was a result of the presence of excessive moisture that had adverse effect by diluting the bonding material and reduced its effectiveness to bond adequately to the resin composite. (Swift, 1998).
Swift (1998) showed the collapse of the essential collagen network in etched and dried dentine. This collapse inhibits the further penetration of the primer and bonding agents, compromising bonding. Moist techniques are therefore advocated to prevent this collagen collapse and increase bonding.

**Figure 2: Schematic diagram of bonding to dentine tubules using a strong self-adhesive bonding agent (Sarr et al., 2010)**

**Abbreviation:** HAp- Hydroxyapatite crystal

Adhesive agents used to facilitate composite adhesion to tooth structures are also technique sensitive, especially during their application onto dentine surfaces (fig 2). Ideally, adhesive agents should be biocompatible, should have adequate bond strength, and should bond to both enamel and dentine structures without any difficulties (Kamble et al., 2015; Pongprueksa et al., 2016). Demineralization of dentine structures by cariogenic bacteria exposes the dentine surface to developing a smear layer, which may affect adhesion (Trivedi et al., 2014).
1.2.1.3 Smear layer

The smear layer is a 2μm layer consisting of chips of cut enamel and dentine, amorphous bacterial debris and blood (Trivedi et al., 2014). It results from bacterial contamination and the cutting of hard tissue with hand or rotary dental instruments. This layer occludes the dentinal tubules thereby affecting adhesion (Trivedi et al., 2014). The removal or modification of this smear to improve adhesive strength of resin materials is thus debatable.

Certain bonding agents require the complete removal of the smear layer by acid etching the dentine surface to create a demineralized surface and opening of the dentinal tubules for more effective adhesion. When the smear layer was completely removed, dentine sensitivity and material shrinkage increased (West et al., 2014), whereas modifying the smear had simplified the clinical handling of these materials but had compromised the bond strength to dentine. (see table 1.2) Thus the quality of the smear layer, and the rinsing and drying of the bonding agents played a role in determining the bond strength of dentine bonding agents to tooth structure (Koibuchi et al., 2001).

1.2.2 Dentine bonding agents

Table 1.2 outlines the evolution, composition and bonding mechanisms to tooth structure
Table 1.2. Evolution of bonding agents (Generations 1-7)

<table>
<thead>
<tr>
<th>Generation (time)</th>
<th>Type</th>
<th>Components, bonding and their failure</th>
<th>Smear layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st (1950)</td>
<td>Total-etch; Separate bottles</td>
<td>It consists of a strong acid etch (85 % Phosphoric acid) (Swift, 1998) and a separate adhesive; No Primer was included in the bottle. The adhesive consists of N-phenylglycine and glycidyl methacrylate (NPG-GMA). Bonding was by the bifunctional resin molecules to the calcium ions on the hydroxyapatite crystals on enamel. Bond failure occurred under moisture contamination causing further composite leakage. Additional dentine re-etching did not increase the bonding interface (Kugel et al., 2000). Therefore bonding was to enamel only.</td>
<td>Smear layer was removed</td>
</tr>
</tbody>
</table>
| 2nd (1970)        | Total-etch; Separate bottles  | It consists of a reduced acid etch (>37% <70%) and sometimes uses 20% hydrogen peroxide etchants. They have three different types of adhesive systems:  
1. Etched tubule dentine bonding agents consist of 25% critic acid and ethylmethacrylate;  
2. Phosphate ester dentine bonding agents consist of phosphate esters bound to BIS-GMA  
3. Polyurethane dentine bonding system consists of polyurethane involving di-isocyanate. All these adhesives had also halophosphorous esters of unfilled resin such as bisphenol-A-glycidyl methacrylate (Bis-GMA) and or Hydroxylethyl methacrylate (HEMA)  
There was no Primer included for bonding. Bonding was therefore by ionic bond to calcium on the tooth structure by the | Smear layer left intact                                                      |
formation of chlorophosphate crystals. Although this may have increased their bond strength to tooth structures, it could not prevent moisture contamination. Additional dentine re-etching did not increase the bonding interface and bonding was to enamel only (Kugel et al., 2000). Therefore the second generation didn’t replace the first generation bonding agents but served equally well under clinical settings (Douglas, 1989; Charlton, 1996).

| 3rd (1980-1990) | Total-etch; All separate bottles. | It consists of an etchant (10% maleic acid which was changed in 1994yr. to 35% phosphoric acid), Primer (6% phosphate penta-acrylate; 30% HEMA and 64% ethanol) and adhesive (same composition as the primer with HEMA). Moisture contamination had been controlled however, the primers cured quickly inhibiting bonding to composite materials. Bonding to dentine was also less effective as compared to bonding to enamel (Kugel et al., 2000). | Smear layer was partially removed and/or modifies the smear layer |
| 4th (early 1990 to 1992) | Total-etch; Multiple bottle | It consists of an etchant (40% phosphoric acid for 60 seconds) (Swift, 1998), primer (hydrophilic monomers and HEMA) (Kugel et al., 2000) and adhesive (same composition as the primer) (all separate bottles) Bonding was by the formation of resin tag (micromechanical bonding) called hybridization of the dentine. Bonding to both enamel and dentine was thus achieved. Bond failure occurred by the acid etch that dissolved the dentine tubules. This had increased the water within the tubular that prevented further adhesion. It also caused the collagen network to collapse (Kugel et al., 2000) and increased post-operative hypersensitivity. | Complete removal of the smear layer |
| 5th (1998) | Total- etch; Two bottles | It consists of an etchant (35-37% phosphoric acid for 15-20 seconds) and separate Primer-Adhesive (1 bottle) containing hydrophilic (PENTA, methacrylate phosphates, ethanol and acetone) and resin hydrophobic monomer (Kugel et al., 2000). Bonding was by micromechanical adhesion. Even though HEMA was completely removed, poor adhesion against the enamel margins were evident (Charlton, 1996). | Complete removal of the smear layer |
| 6th (type 1) (between the late 1990 and early 2002) | Self-etching acidic primer and separate adhesive | It consists of an acidic primer (37% phosphoric acid) into 1 bottle and a separate adhesive consisting of ethanol, acetone and water (the other bottle). Bonding is by micromechanical adhesion. It produced a thick hybrid layer that increased the bond strength. It did however, fail in colour and didn’t set by light curing using a plasma arc curing light. It was recommended for use with certain resin cements and had a better seal against dentine than to enamel. | Modified the smear layer |
| 6th (type 2) (between the late 1990's and early 2002's) | Self-etching adhesive One bottle | It consists of an acidic primer adhesive consisting of 34% phosphoric acid, methacrylate phosphate, ethanol, acetone and water (1 bottle). The bond failed onto moist and dry dentine surfaces because it produced a very thin hybrid layer. The weak infiltration of the resin monomers reduced the bond strength of this agent. Therefore it was not recommended for used with self-cured composite cores and resin cements. | Modified the smear layer |
| 7th (Type1) (Late 2002) | No-mix: Self-etching adhesive One bottle | Acidic primer Adhesive (1 bottle)(see below) | Modified the smear layer |
Abbreviations:
NPG-GMA: N-phenylglycine and glycidyl methacrylate;
Bis-GMA: bisphenol-A-glycidyl methacrylate (Bis-GMA);
HEMA: Hydroxylethyl methacrylate;
PENTA: Phosphate penta-acrylate

The first and second-generation dentine bonding agents (table 1.2) were developed by 1970’s had provided a large dentine-wetting surface. However, they had overall poor clinical performance (Swift, 1998). The third generation agents were introduced (1980’s) with the aim of reducing the marginal leakage and improve the retention rate of the previous bonding agents. Unfortunately, these bonding agents could not prevent marginal leakage as these bonding agents were hydrophobic and had shown failures as a result of water within the tubules counteracting this bonding system. (Swift, 1998).

Nakabayashi (1982) then described the formation of a hybrid layer, which is the penetration of hydrophilic monomers in acid-etched dentine that improved dentine adhesion. This was then followed by Fusayama (1992), who introduced the total etch technique. This involved a three-step enamel-dentine preparation system that consisted of an acidic conditioner, primer and bonding agent that improved tooth adhesion.

These developments led to the 4th generation of bonding agents (table 1.2) when the bonding to dentine became more successful. According to Sano et al (1999), the bond strength of composite to the dentine surfaces was as strong as that to enamel. This was the result of a better bond to interdental tubules following the complete removal of the smear layer. HEMA (hydroxyethyl methacrylate) was still included in these bonding agents. This served to hydrate the dentinal tubules after aggressive etching, which facilitated effective adhesion onto demineralised dentine (Katz et al, 2001; Hitmi et al., 2002). However, HEMA was later discontinued from the 5th generation agents as it was found to be toxic to human tissue (Mine
et al., 2008). Dentine hypersensitivity had therefore increased after the smear layer was removed (West et al., 2014).

The 5th and 6th generation bonding agents (table 1.2) were also designed to simplify the steps by combining either acid and primer or bonding agents or all three into a one bottle system. However, they had poor adhesion to moist and dry dentine surfaces (Mohan et al., 2005; Bassir et al., 2012).

The 7th generation moved to a single–step application of etchant, primer and bonding material called self-etching adhesive agents. The reason for the change was that when separate etch–primer adhesives were used, several leakage pathways at the adhesion interface occurred, and breakdown of the collagen fibres surrounding the dentinal tubules reduced the adhesion to composite (Van Meerbeek et al., 1994; Hashimoto et al., 2000). De Munck et al (2005) believed that the three steps of etch, rinse and adhesive had remained the gold standard in terms of adhesion durability. These techniques were effective for enamel bonding (Mortazavi et al., 2012) but not for dentine bonding. Failure occurred from the adhesive agent’s inability to penetrate the dentine tubules (Schneider et al., 2000; Lopes et al., 2002).

The residual solvent of a bonding agent, which when placed on the dentine surface of a tooth, may also affect adhesion. The recommendation of air-drying these solvents using a 3-in-1 dental syringe was considered to increase adhesion (Marsiglio et al., 2012; Lau et al., 2014). Researchers claimed that the amount of air-pressure applied over the excessive solvent would determine the best clinical performance of the bonding agent (Marsiglio et al., 2012; Lau et al., 2014). The clinical performance of bonding to dentine at room temperature is also dependant on the type of material used (Marsiglio et al., 2012).

The 7th generation self-etching agents such as Scotchbond Universal contain the following valuable components: methacryloxydecyl phosphate monomers (MDP);
dimethylaminododecyl methacrylate; copolymers; filler particles; ethanol; water; initiators and silane. The dimethylaminododecyl methacrylate and copolymers will modify the smear by their low acidic potential (Söderholm et al., 2005; Penmetsa et al., 2014; Münchow et al., 2015) provide dentinal surface protection, maintain an adequate adhesion (Mortazavi et al., 2012) and dentinal tubular patency (Söderholm et al., 2005; Söderholm et al., 2008; Goswami et al., 2014; Suzuki et al., 2015). The filler particles provide dimensional stability reducing the polymerisation shrinkage of the resin monomers (Söderholm et al., 2005; Söderholm et al., 2008). The ethanol and water provide a base-solvent system (Söderholm et al., 2005; Söderholm et al., 2008). The silane allows the adhesive to chemically bond to glass ceramic surfaces without using a separate ceramic primer (Söderholm et al., 2005; Söderholm et al., 2008).

These agents can also hydrate the dentinal tubules and sustain the collagen fibres from total collapse (Söderholm et al., 2008). Manufacturers claimed that these self-adhesive agents have a total etch and self-etch mode (LeBlanc, 2013). There is minimum postoperative sensitivity, and they are a reliable source of hydrophilic bonding to dentine (Söderholm et al., 2005; John et al., 2015). Bisphenol-A-glycidyl methacrylate (Bis-GMA) in self-etching adhesives has replaced the HEMA as a safer component in the adhesive agent (LeBlanc, 2013).

Dentine bonding agents work optimally well under ideal conditions and require a contaminant free tooth surface. Oral contaminates such as saliva and blood during subgingival cavity preparation are difficult to control causing further restoration failure. The need to investigate the efficacy of the new self-etching adhesive onto contaminated cavities was thus necessary.
1.3. Contamination

For an adequate adhesion to occur, cavity preparation techniques require tooth cut surfaces to be contaminant free. Persistent and destructive types of oral microbes cause an accelerated rate of caries production more commonly on the dentine than to enamel surface leading to a further composite adhesion failure (Li et al., 2014).

Ideally, the placement of a rubberdam should always be considered to minimize the adhesive failure rate of composite restorations by contamination. However, practically this may not always be possible. Deep cavity preparations, where it is impossible to place a rubberdam subgingivally, are prone to contamination that may lead to failure at the bonding interface between the adhesive and dentine surfaces (Yoo et al., 2006).

Contaminants can prevent the bonding agent from penetrating the dentine tubules effectively. However, the question to dry or not to dry the moist dentine surfaces before the application of the dentine bonding agent had to be evaluated, in order to choose the correct bonding agent with the greatest moisture resistance and strength. Lightly drying the dentine after fluid contamination was assumed to reduce contamination and increase adhesion (Yoo et al., 2006, Mathews et al., 2008), as compared to over drying the dentine, which caused the collagen to hydrolyse, increased the intra-dentinal fluid movement and sensitivity (Hashimoto et al., 2000). Excessive water loss under ambient conditions had also caused a rough dentine surface to form that impeded adhesion (El Feninat et al., 2001).

Mathews (2008) stated that air drying the self-etching adhesive had removed the excessive water droplets on the surface of the dentine, prolonged the adhesion onto dentine and prevented collagen collapse. Excessive drying should be avoided, even though it had retained
small amounts of fluid to prevent collagen collapse, the adhesion after the initial dehydration had been debatable (Liu et al., 2011).

1.3.1. **Contaminants** include the following five main categories:

1.3.1.1 Saliva  
1.3.1.2 Vapour  
1.3.1.3 Gingival crevicular fluid  
1.3.1.4 Blood  
1.3.1.5 Water  
1.3.1.6 Water from the dental unit waterlines

**1.3.1.1 Saliva**

Saliva consists of 600-1000ml of fluid per day and includes electrolytes, antibacterial enzymes, calcium phosphate crystals and immunoglobulins secreted by salivary glands (Nance, 2013; Van Meerbeek et al., 1994; Sano et al., 1999). Salivary flow rate is increased by the gustatory reflex under the influence of the olfactory, proprioceptors and ophthalmic receptors that are involved in seeing, smelling, feeling and tasting the foods. Saliva has the ability to transport cariogenic bacteria and pathological fungal strains (Zhang et al., 2015). For the general dentist, saliva contamination continues to be a challenge when placing composite restorations reducing the microtensile bond strength (Hitmi el al., 1999; Aboushelib, 2011). Rubber dam is the isolation method of choice, but cotton rolls are often used to isolate cavity preparation from saliva. The use of cotton rolls was described by Aboushelib (2011) when failure occurred in placing the rubberdam correctly in difficult subgingival cavity preparation. They have been described to isolate the cavity preparation from contaminants (Sano et al., 1999). Failure to bond to the dentine surface by fluid
contaminants produced additional calcium phosphate crystals that affected the bond to resin composites (Van Meerbeek et al., 1994; Sano et al., 1999).

Yoo (2006) suggested that saliva contamination onto dentine had shown significant reduction in bond strength. Most bonding studies have been undertaken on bovine enamel surfaces, showing increased enamel-bonding failures (Turk et al., 2007; Jiang et al., 2010; Shimazu et al., 2014). Further studies were needed to assess the effect of saliva contamination on the bond strength to human dentine.

1.3.1.2 Vapour

**Intraoral vapour (Humidity)**

Humidity is defined as the amount of water vapour in the air and can be influenced by the temperature changes in the oral cavity (Longman et al., 1987; Saraiva et al., 2015). Dentine bonding agents under high humidity conditions have shown adhesive failure.

The intraoral humidity is influenced by the relative humidity and temperature in the dental surgery. This can be controlled by the application of a rubberdam and air conditioning (Plasmans et al., 1993). The high humidity in the oral cavity can affect enamel and dentine adhesion by vapour settling onto prepared cavity preparations (Cacciafesta et al., 2003; Li et al., 2014)

**Mercury particles**

The removal of old amalgam fillings causes the mercury particles to occlude the dentinal tubules compromising the penetration of the bonding agent. This has been implicated as the cause of adhesive failure between the tooth and bonding agent in these cases (Sepetcioglu et al., 1998). According to Sepetcioglu (1998), evidence suggested that the cavity should be
enlarged to remove the occluded residual mercury particles to accommodate the penetration of the bonding agents onto the dentine surface.

1.3.1.3 Gingival crevicular fluid

Cavity preparation is often close to the gingival crevices. The effusion of crevicular fluid with antibacterial enzymes is increased during active periodontal disease and inflammation around the gingiva (Masada et al., 1990). Crevicular fluid is also increases after the application of phosphoric acid within the self-etching adhesives onto the soft tissues. The acid will release matrix metalloproteinase enzymes (MMP) from the cells in the gingival crevices to the crevicular fluid which breaks down the collagen network in the hybrid layer, further affecting the bond to tooth structures and composites (Moon et al., 2010).
3.1.4 Blood

Bleeding is a common complication of tooth preparation, particularly with large and/or subgingival cavities. This has been implicated in reduced bond strength. Red blood cells contain haemoglobin that carries oxygen from the lungs to the rest of the body and carbon dioxide away from the body to be excreted from the lungs. Iron minerals further enrich the haemoglobin structure and assist in maintaining normal haemostasis. White blood cells contain important cells such as lymphocytes, monocytes, eosinophils, basophils and neutrophils. These cells provide defence against pathogenic bacteria and host cells immunity (auto-immunity). The plasma is a major component consisting of water (primarily) which contains electrolytes and antibacterial protein such as albumin. Albumin is a major macro-protein that prevents the blood vessels from collapse or clogging. Platelets provide anti-coagulating properties. The oral soft tissue is highly vascularized. Blood contaminants were never subdivided into its individual constituents under clinical trial studies to evaluate their effect on individual bonding failures (Tachibana et al., 2011). Freshly drawn blood should be collected in anti-coagulating vials (Chang et al., 2010, Tachibana et al., 2011) or blood samples should be collected via diabetic pin prick protocol and applied directly to exposed dentine surfaces (Itoh et al., 2000).

A common blood related bonding failure was haemorrhage from the gingival col area in deep cavity preparations that are difficult to control even when using ferric sulphate solution to control excessive bleeding (Kilic et al., 2013). Enamel contamination required additional rinsing of the blood and re-etching before the application of the composite. This had a significant reduction on the bond strength implying the need to further prepare the cavity margins (Faltermeyer et al., 2007). Contamination of dentine with blood hinders the
penetration of the bonding agent into the dentine tubules (Zortuk et al., 2010; Güngör et al., 2013).

### 1.3.1.5 Water

Water from the 3-in-1 syringe, the dental fast handpieces and scaler tips produces splatter that contains the bulk of bacteria and water particles that settle into cavity preparations (Dahlke et al., 2012). These can cause adhesion failure between the bonding agent and the tooth substrate. Water sanitation protocols require the need to assess the water source that enters into the patients’ oral cavity that passes through our dental unit.

### 1.3.1.6 Water from the dental unit waterlines

Dental unit waterlines (DUWL) connect water from public reservoirs to the handpieces, triplex syringes and water bowls through narrow bore flexible plastic tubing. Microorganisms have been shown to enter through the dental equipment, and are transmitted via aerosols and splatter into the patient’s oral cavity. These have increased incidence of Legionella, Staphylococcus aureus and Pseudomonas aeruginosa infections (Galal-Gorchev, 1996; Coleman et al., 2009; O’Donnell et al., 2011; Tuladhar et al., 2012; Salvia et al., 2013; Sorlini et al., 2014, Watanabe et al., 2016).

Oral stable biofilms consist of organised commensal polymicrobials embedded with the extracellular matrix of the mucosa and dental tissue (Do et al., 2013). Dental disease like caries causes bacteria to accumulate leading to an unstable biofilm (Do et al., 2013). Dentist can create this unstable environment by drilling tooth structures and contaminating tooth surfaces with blood.

Biofilms that contain resistant microorganisms are killed by chlorine dioxide solutions within the dental unit waterlines (Meiller et al., 1999; O’Donnell et al., 2011; Patel et al., 2012; Patel
et al., 2016). There were limited studies of chlorine dioxide solution been used within DUWL! Chlorine dioxide solution was shown in a SA study to kill 99.95% of microorganisms from the DUWL tubing (Patel et al., 2016) and disinfects the alginate impressions (Rweyendela et al., 2009). Chlorine dioxide solution can therefore provide a necessary dental surface disinfectant and is available at a potable concentration in our underlying water pumps (Simpson et al., 1993; Galal-Gorchew, 2009; Csilla et al., 2009; Noszticzius et al., 2013; Watamoto et al., 2013; Sorlini et al., 2014). A major concern of the use of chlorine dioxide in drinking water is that it can dissociate into a chlorite and chlorate which, if left standing will become contaminated (Lubbers et al., 1982). If chlorine dioxide did not dissociate then it could be used as a powerful disinfectant solution (Lubbers et al., 1982; Porteous et al., 2009; O’Donnell et al., 2011; Rweyendela et al., 2009). To prevent chlorine dioxide from dissociation the pipelines need to be constantly monitored and cleaned (McDowell et al., 2004; Szymańska et al., 2008, Patel et al., 2016). No studies on the effect of chlorine dioxide on bond strength have been undertaken.

### 1.3.2 Rubberdam

Contamination of prepared surfaces can be prevented by the application of a rubberdam. Rubberdam has benefits in restorative and endodontic treatment and continue to be taught in dental schools (Clark et al., 2001; Ahmad, 2009). Rubberdam isolation provided the advantage of “improved operator access and visibility, minimization of airborne debris and aerosols, and patient safety” (Hill et al., 2008). Research has shown that not many dentists preferred this method of tooth isolation due to it being time consuming to place (Hill et al., 2008; Kapitán et al., 2011).
1.4. Conclusions

Composites have become an essential material for the restoration of teeth. They do, however require careful manipulation as the material is technique sensitive. Good isolation and tooth preparation protocols need to be observed. Nanocomposites have shown to be more effective with Scotchbond Universal self-etching adhesive agents (Froggett et al., 2014).

Their success is dependent on a strong and long lasting bond to the underlying tooth material. This has been well researched and established with regard to enamel. The quest to achieve similar bond strength to dentine has been a significant challenge due its complex microstructure and biology. In order to overcome these obstacles manufacturers and researchers produced seven generations of bonding agents since the 1970’s in search of an adequate long-term bond to dentine.

Self-etching materials have been claimed to be a significant advance in bonding technology and were developed in order to simplify the growing complexity of the dentine bonding protocols. However, their bond strength to enamel and dentine is not as good as the previous generations. Manufacturers claimed that these materials bonded successfully to moisture contaminated enamel surfaces with remineralisation of the apatite crystals that had been confirmed in a number of studies (Cacciafesta et al, 2003; Liu et al., 2011; Suryakumari et al., 2011).

They further claimed that good dentine bonding could also be achieved on moist, contaminated surfaces using the same chemistry (3M ESPE, St Paul, USA). This has, however, not been sufficiently tested particularly with oral contaminants of blood and saliva on human dentine.
Bacteria are unavoidable inclusions in the oral contaminants. Chlorine Dioxide has been proven to be an extremely effective biocompatible disinfectant, but its effect on bonding agents has not been established.

This research project therefore sought to establish the effects of oral contaminants on the bond strength of self-etching adhesives to human dentine as most studies have been done using bovine and orthodontically treated enamel teeth (Itoh et al., 2000; Zeppieri et al., 2003; Cacciafesta et al., 2003; Faltermeier et al., 2007; Sayinsu et al., 2007; De Alexandre et al., 2008; Vicente et al; 2009; Maia et al., 2010; Rüttermann et al., 2013; Sfondrini et al., 2013; Prasad et al., 2014; Purushothaman et al., 2015).
CHAPTER 2

AIMS AND OBJECTIVES

2.1 Aim

To compare the shear bond strength of Filtek Supreme XTE composite bonded with Self Etching Universal Scotchbond Adhesive to uncontaminated cut dentine, with cut dentine contaminated with saliva, blood and disinfectant.

2.2 Objectives

1. To measure the shear bond strength of a nanocomposite to uncontaminated cut dentine.

2. To measure the effect of contamination by blood, saliva, and a disinfectant spray containing chlorine dioxide on the shear bond strength of a nanocomposite to cut dentine.

3. To determine the nature of the bond failure.

2.3 Null Hypothesis

There is no difference in the shear strength between any of the conditions of the study.

2.4 Ethical Clearance (Appendix B)
3. Method

3.1. Pilot study

A pilot study (with ethical clearance (Appendix B) was carried out using twelve caries free freshly extracted premolar teeth placed in a 0.5 % Chloramine T solution. The study was undertaken according to a standardized proposed protocol from the outcome variables (contaminants) was based on a significant level of 5 %, power of 80%, and the effect size of main sample size calculated from the pilot study was nine teeth. These nine teeth were randomly selected and placed into the same four groups of this main study.

3.2. Methods and Materials

Thirty-six freshly extracted caries-free premolar teeth were used for the study. Each tooth was then placed in 0.5% Chloramine T solution (Measured at 0.01mg of powder added to 1litre glass bottle containing 1 litre of distilled water) for 4 hours (Hitmi et al., 1999, Leevailoj et al., 2007) in order to disinfect them. Each tooth was then cleaned of any debris with a toothbrush and thereafter placed in a saline solution at room temperature for the closest possible concentration relation to oral saliva. The teeth were used within a month after extraction and controlled by the researcher. The 0.5% Chloramine T solution was changed every 1.5 weeks for the duration of the study to remove the contaminated solution in accordance with other accurate studies (Hitmi et al., 1999, Rweyendela et al., 2009, Patel et al., 2016).
The teeth were secured in a stainless steel mounting ring (Bencore) measuring 1.2 cm in length and 1.5 cm in diameter (Fig 3.1) using a clear self-curing acrylic resin to stabilize the tooth (Melodent, USA) (Fig 3.2). Once the acrylic resin was polymerised, the sample was immersed in a saline solution and placed in an incubator (220v, Labotec, South Africa) at 37°C-37.5°C for 24 hours to standardize the resembles of oral tissue temperature (Dursun et al., 2011).

Figure 3.1 Stainless steel rings (Bencore) of 1.5cm by 1.2cm on a pink baseplate wax

Figure 3.2 Melodent self-curing acrylic poured into the rings (salt and pepper technique)

The enamel surface of the premolars were ground down using a laboratory Pro-trim 1725 Hertz grinder using 600 grit silicon carbide fine grinding paper to remove all occlusal enamel and expose the dentine surface of each tooth (Hitmi et al., 1998; Dursun et al., 2011, Suryakumari et al., 2011, Koppolu et al., 2012, Munaga et al., 2014). The sample was then re-immersed in
the saline solution and incubated at 37°C - 37.5°C for 24 hours to maintain a close resemblance of oral tissue temperature (De Alexandre et al., 2008, Dursun et al., 2011).

The self-etching adhesive agent Scotchbond™ Universal Adhesive (3M ESPE, St Paul, USA) was then applied with a microbrush to the cut dentine for 20 seconds, dried for 5 seconds using a 3-in-1 dental syringe and light cured for 10 seconds by using calibrated Elipar S10 LED curing light at an standardized set intensity of 430 nm (calibrated by the suppliers) (3M ESPE, St Paul, USA) in accordance with the manufacturer’s instructions (Fig 3.3).

![Figure 3.3 Scotchbond universal applied 20 seconds (A), dried for 5 seconds (B) and light cured 10 seconds perpendicular to the tooth surface (C).](image)

The composite, Filtek Supreme XTE (3M ESPE, St Paul, USA) was bonded to the adhesive through an Ultradent bonding mould attached onto an Ultradent bonding clamp (Ultradent, South Jordan, UT, USA) to establish identical composite dimensions for all samples. The curing light was then applied at a standard perpendicular angle to the tooth surface for 40 seconds according to the manufacturer’s instructions (Fig 3.4).
Figure 3.4 A: Ultradent bonding clamp and mould at 0.25mm diameter; B: Filtek Supreme XTE A3.5 composite is placed through the mould and C: light cured.

Human blood and saliva was collected from the researcher. The researcher’s blood was collected by the application of pricking the third left finger with an Accu-Check safe tip and transferred to a microbrush of a diameter of 1mm (Fig 3.5).

Figure 3.5 Accu-check safe tip (A) used to prick finger and blood is collected with 2mm microbrush (B)

Saliva was collected by applying a 1mm microbrush lingually and adjacent to the 2nd mandibular molar closest to the lingual salivary duct (Whartons’ duct). A ten part per millions (10 ppm) potable solution of chlorine dioxide (Patel et al., 2016) was applied with an insulin syringe of 6mm gauge and dried for 5 seconds, according to manufacturer’s instructions.
3.2.1 Experimental types and methodology

**Group 1 (control group)**

Instructions: The prepared dentine surfaces were air dried perpendicular to the exposed surface for 5 seconds (Fig 3.3 B). Scotchbond™ Universal adhesive was applied according to the manufacturer’s instructions. There was no contamination of the dentine and the bonding agent was applied and light-cured, and the composite added.

**Group 2 (Human blood contamination at 5 seconds)**

Instructions: Blood contaminate was applied for 5 seconds with a microbrush, air dried for 5 seconds and the bonding agent was then applied and light-cured. Thereafter it was bonded to the composite.

**Group 3 (Human saliva contamination at 5 seconds)**

Instructions: Saliva contaminate was applied for 5 seconds with a microbrush, air dried for 5 seconds and then the bonding agent was applied and light-cured. Thereafter it was bonded to the composite.

**Group 4 (Chlorine dioxide contamination at 5 seconds)**

Instructions: One ml of chlorine dioxide disinfectant was applied for 5 seconds through an insulin syringe; air dried for 5 seconds and then the bonding agent was applied and light-cured. Thereafter it was bonded to the composite with the same standard techniques.

All prepared teeth were placed in a saline solution and stored for 24 hours in an incubator at 37°C - 37.5°C. Intragroup randomisation of each tooth in its stainless steel ring was carried out using a random number table. Each tooth was then placed in a test base clamp on an Instron testing machine, sheared by a 2kN load cell at a cross-head speed of 0.5mm/second.
according to International standard organisation (ISO) specification no. 11405 on shear bond standards (Dental Materials, 2003, Sirisha et al., 2014). The Instron machine (Model number 3344K6219) using blue hill 2 software was used to determine shear bond test results as illustrated in Fig 3.6.

Figure 3.6  A: The Shear bond apparatus; B: The shearing off the composite plug from the dentine cut surface
A stereo microscope (Nikon SMZ, Johannesburg, South Africa) was used to assess the modes of failure (adhesive or cohesive) at 20 X magnification; brightness: 50 ± 2 and intensity: 80 ± 2 (Schneider et al., 2000) and revealed:

1. Failure of the dentine bonding agent to the dentine was categorized as Adhesive failure (BD)
2. Failure of the dentine bonding agent to the composite was categorized as Adhesive Failure (BC)
3. Failure within the composite was categorized as Cohesive Failure (C)
4. Fracture within the dentine was categorized as Dentine failure (D)
5. Exposure at the pulp was categorized as Pulp failure (P)
Key: DBA: dentine bonding agent; BD failure site: Bond to dentine failure site

Figure 3.7 Stereo microscope analysis of the failure of the dentine bonding agent to the dentine was categorized as Adhesive Failure (BD). Magnification: 20:1

Figure 3.8 Stereo microscope analysis of the failure of the dentine bonding agent to the composite was categorized as Adhesive Failure (BC). Magnification: 20:1
Figure 3.9 Stereo microscope analysis of the failure within the composite was categorized as Cohesive Failure (C). Magnification 20:1.

Figure 3.10 Stereo microscope analysis of fracture at the dentine and pulp was categorized as Dentine (D) and Pulp Fracture (P). Magnification: 20:1
3.3 Data analysis

The pilot study data were not considered for statistical data analysis. The outcome of this study was to measure the differences in the different groups’ bond strengths. For each group, the results were tabulated showing mean and standard deviations. Each group was compared to the control group data and the methods of failure were assessed. A 5% significance level was employed throughout the study. The data was carried out using the SAS software (SAS institute inc, USA) using a one-way Analysis of Variance (ANOVA) test.
4.1. Pilot study

A pilot study was conducted to determine the sample size of this research. Sample size estimations were based on a significance level of 5% and a power of 80%. The calculated total sample size of the main data was 36; i.e. 9 samples per treatment group.

4.2. Main data analysis

4.2.1 Distribution of data

Figure 4.1 A histogram of the distribution of the bond strength data.

The histogram (Fig 4.1) denotes a symmetrical distribution of the bond strengths values that were considered normal.
4.2.2. Results

The univariate statistics (mean, standard deviation, median, interquartile range) for the shear bond strength are shown in Table 4.2.

Table 4.2 Univariate statistics of the main data

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of teeth</th>
<th>Mean (MPa)</th>
<th>Standard deviation (Std dev)</th>
<th>Minimum (MPa)</th>
<th>Maximum (MPa)</th>
<th>Relative standard deviation (RSD) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>9</td>
<td>13.2</td>
<td>6.4</td>
<td>4.2</td>
<td>22.5</td>
<td>49</td>
</tr>
<tr>
<td>Group 2</td>
<td>9</td>
<td>5.4</td>
<td>4.0</td>
<td>0.3</td>
<td>11.1</td>
<td>74</td>
</tr>
<tr>
<td>Group 3</td>
<td>9</td>
<td>11.6</td>
<td>4.4</td>
<td>5.7</td>
<td>18.7</td>
<td>38</td>
</tr>
<tr>
<td>Group 4</td>
<td>9</td>
<td>14.5</td>
<td>5.1</td>
<td>7.2</td>
<td>20.7</td>
<td>35</td>
</tr>
</tbody>
</table>

Key:
Group 1 = Cut uncontaminated dentine (Control)
Group 2 = Blood contamination
Group 3 = Saliva contamination
Group 4 = Chlorine dioxide disinfectant

The data for each treatment group sample (Table 4.2) had shown RSD variations, particularly for Group 2, at 74% with the largest distribution percentage of bond strength.
Figure 4.2 Box-and-whisker plots: The bottom and top edges of the box indicate the interquartile range (IQR), that is, the range of values between the first and third quartiles (the 25th and 75th percentiles). The marker (●) inside the box indicates the mean value. The line inside the box indicates the median value. The whiskers that extend from each box indicate the range of values that are outside the interquartile range but within 1.5*IQR of the median. Values beyond this range are indicated by markers.

In Fig 4.2, the size of the box plot indicates the range and variation of the bond strength data per group (IQR). Each sample had mean values that were within the interquartile box range (the middle 50%) and none of the values were outliers. Blood samples (group 2) have a mean value superior to the median value within the interquartile range and a smaller spread of the bond strength (IQR) as compared to the other groups. Chlorine dioxide samples (group 4) had a mean value and a box plot higher than any of the other groups.

4.3 Analysis of main study data

The bond strength was calculated by a one-way ANOVA that showed results with
a p-value of 0.0032 was significant. Post-hoc tests using Dunnett’s procedure (Figure 4.3) was used to compare each of the three treatment groups to the group 1 (Control). It showed that the mean bond strength for group 2 (Blood) samples (5.4±3.1 MPa) was significantly lower compared to the group 1 (Control) samples (13.2±5.0 MPa) (values after the ± denote the 95% confidence interval for the mean). The effect size was large (table 4.3). The treatment types and mean bond strength are displayed in figure 4.3.

**Table 4.3 Comparisons between treatments**

<table>
<thead>
<tr>
<th>Treatment Comparison</th>
<th>Difference</th>
<th>Simultaneous 95% Confidence</th>
<th>p-value</th>
<th>Cohen's d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Between Means</td>
<td>Limits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 4 (Chlorine dioxide) – group 1 (Control)</td>
<td>2.266</td>
<td>-2.707</td>
<td>7.239</td>
<td>0.55</td>
</tr>
<tr>
<td>Group 3 (Saliva) – group 1 (Control)</td>
<td>-1.355</td>
<td>-6.328</td>
<td>3.619</td>
<td>0.85</td>
</tr>
<tr>
<td>Group 2 (Blood) – group 1 (Control)</td>
<td>-6.622</td>
<td>-11.595</td>
<td>-1.649</td>
<td>***</td>
</tr>
</tbody>
</table>

*** A large statistical difference.

A comparative analysis of the three groups to group 1 (table 4.3) showed Cohen’s d was larger in the group 2-group 1 samples (1.27) with a statistically significant p-value of 0.0064. The group 4-group 1s’ samples and group 3-group 1s’ samples have p-values of 0.55 and 0.85 respectively.
The inter-group data analysis has a 95% probability to lie within the intervals and are true values in the parameters of the study (fig 4.3). Interpretation of the above graph revealed a higher confidence level for the group 1 (mean value at 13.2) and group 4 (mean value at 14.5) samples as compared to the other groups.
Table 4.4 The combined statistical data of the main study in order of sequencing

<table>
<thead>
<tr>
<th>Date</th>
<th>ID</th>
<th>Run order</th>
<th>Mmt Order (Randomised)</th>
<th>Treatment</th>
<th>Bond strength</th>
<th>Classification of Failure: BD/BC/C/ P</th>
</tr>
</thead>
<tbody>
<tr>
<td>07-Nov-14</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>HB</td>
<td>2.49</td>
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</tr>
<tr>
<td>07-Nov-14</td>
<td>2</td>
<td>1</td>
<td>1</td>
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<td>7.23</td>
<td>D</td>
</tr>
<tr>
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<td>21.86</td>
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<td>07-Nov-14</td>
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<td>2</td>
<td>7</td>
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<tr>
<td>07-Nov-14</td>
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<td>2</td>
<td>SALIVA</td>
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<td>BD</td>
</tr>
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<td>2</td>
<td>5</td>
<td>CHI DI</td>
<td>13.99</td>
<td>D</td>
</tr>
<tr>
<td>07-Nov-14</td>
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<td>9</td>
<td>CONTROL</td>
<td>14.53</td>
<td>BC</td>
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<td>6</td>
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<td>8.52</td>
<td>BD</td>
</tr>
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<td>3</td>
<td>8</td>
<td>CONTROL</td>
<td>16.37</td>
<td>BC</td>
</tr>
<tr>
<td>07-Nov-14</td>
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<td>5</td>
<td>SALIVA</td>
<td>12.76</td>
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<tr>
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<td>2</td>
<td>CHI DI</td>
<td>18.01</td>
<td>P</td>
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<td>07-Nov-14</td>
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<td>9</td>
<td>CONTROL</td>
<td>22.46</td>
<td>BC</td>
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<td>8</td>
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<td>3</td>
<td>HB</td>
<td>4.03</td>
<td>BD</td>
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<td>D</td>
</tr>
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<td>BC</td>
</tr>
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<td>9</td>
<td>HB</td>
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<td>BC</td>
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<tr>
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<td>BD</td>
</tr>
<tr>
<td>07-Nov-14</td>
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<td>7</td>
<td>6</td>
<td>CHI DI</td>
<td>20.41</td>
<td>D</td>
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<td>07-Nov-14</td>
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<td>8.02</td>
<td>BD</td>
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<td>07-Nov-14</td>
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<td>8</td>
<td>CONTROL</td>
<td>11.22</td>
<td>BC</td>
</tr>
<tr>
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<td>8</td>
<td>1</td>
<td>CHI DI</td>
<td>11.33</td>
<td>D</td>
</tr>
<tr>
<td>07-Nov-14</td>
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<td>18.71</td>
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</tr>
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<td>07-Nov-14</td>
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<td>SALIVA</td>
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<td>9</td>
<td>CONTROL</td>
<td>7.14</td>
<td>C</td>
</tr>
<tr>
<td>07-Nov-14</td>
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<td>9</td>
<td>5</td>
<td>HB</td>
<td>11.13</td>
<td>BD</td>
</tr>
<tr>
<td>07-Nov-14</td>
<td>9</td>
<td>9</td>
<td>3</td>
<td>CHI DI</td>
<td>17.85</td>
<td>D</td>
</tr>
</tbody>
</table>
The association between treatment and failure location was determined by Fisher’s exact test. The requirements for the chi-square test were not met (Table 4.4). However, the samples were randomised for measurement sequence in the Instron machine.

4.5. Stereo microscopic analysis of the mode of failure

Main study data:

The treatment types and percentages are discussed below.

![Figure 4.4 Percentages of teeth versus the four treatment groups](image)

**Key:**

**BC:** Bonding agent to composite failure (Adhesive failure)

**BD:** Bonding agent to dentine failure (Adhesive failure)

**C:** Failure within the composite (Cohesive failure)

**D:** Fracture within the dentine (Cohesive failure)

**P:** Exposure at the pulp

Thirty-six samples were examined under the stereo microscope with each group exhibiting failure sites (Fig 4.4). Group 2 and 3 samples had most of failure at the bonding agent to dentine level (BD) as compared to the group 1 that had failure between the bonding agent to composite level (BC). BD and BC failure sites were related to adhesion failure. Group 4
samples had shown a variation of failure sites and mostly cohesive fracture within the dentine (D). Consequently, a very strong association had existed between the treatment type and modes of failure as shown in figure 4.4.
CHAPTER 5

DISCUSSION

Adhesive dentistry has changed the future of dentistry by providing good aesthetic restorations through the micromechanical bond of the resin monomer with the tooth structure (Bhatia et al., 2015). Numerous bonding agents have been developed to improve workflow and the quality of adhesives and composite restorations (Kamble et al., 2015). However, moisture contamination continues to be a problem, leading to bonding failure and highlights the need for effective control measures like the isolation with a rubber dam (Bücher et al., 2015). Contaminants have an influence on the bond strength and could adversely affect the final outcome of the restoration. Microleakage and postoperative sensitivity were thus evident if these contaminants were not removed (Bhatia et al., 2015). Bonding to contaminated dentine surfaces (LeBlanc et al., 2013) has further prompted the need for this study, to determine the effects of dentine contamination on the shear bond strength of a self-etching adhesive and a nanocomposite.

In this study, the shear bond strength of Universal Scotchbond self-etching adhesives was bonded to three contaminants, namely saliva, blood and chlorine dioxide disinfectant onto prepared dentine surfaces and modes of failure analysed under a stereo-microscope. The primary objective was to establish the shear bond strength of the self-adhesive bonding agent to the three contaminates onto cut dentine. Several authors have collectively acknowledged the technique sensitivity of applying these self-adhesive agents onto dentine as compared to enamel (Söderholm et al., 2008; De Carvalho Mendonça et al., 2010; Koppolu et al., 2012).
The manufacturers claim that the seventh generation self-etching bonding agents could maintain bond strength in the presence of contamination and bond to moist contaminated surfaces of both enamel and dentine (3M ESPE, St Paul, USA, 2010).

The results, however, (Table 4.2 and 4.3) confirmed that blood contamination (group 2) caused a significant reduction in bond strength (p=0.0064 and RSD=74%) as compared to the control (group 1). This was likely due to an increase in the plasma proteins of blood that interfered with the bond strength of the bonding agent (Chang et al., 2010, Koppolu et al., 2012) or the blood prevented the monomer based bonding agents from penetrating the dentine collagen network (Kuphasuk et al., 2007; Arslan et al., 2013). Bleeding sites could be controlled by the application of haemostatic agents like Viscostat and Viscostat Plus before the application of this type of bonding agents (Ebrahimi et al., 2013). Blood and moisture control by the appropriate use of haemostatic agents and isolation such as rubberdam could limit contamination. Further research is needed to quantify how these agents may or may not affect the bonding agent to dentine surfaces (Ebrahimi et al., 2013).

The results show an insignificant difference in bond strength between the saliva contamination group and the control group (p-value: 0.85). This supports the claims of the manufacturers (3M ESPE, St Paul, USA, 2010) that adequate bond strength could be achieved with saliva contamination in the absence of blood.

Some authors claim that the salivary glycoproteins should inhibit the bonding of the resin monomers (Fritz et al., 1998; Hitmi et al., 1998; Munaga et al., 2014), while others believe the application time of the bonding agent would determine whether salivary bonding failure occurred before or after the bonding agent was applied (Cacciafesta et al., 2003; Turk et al., 2007; Dursun et al., 2011; Rothmund et al., 2015). This study supports those of El-Kalla (1997) and Yazici (2007), showing no sensitivity to saliva contamination. West et al (2014)
postulated that this bonding agent probably inhibited the protective function of the salivary enzymes that were claimed to break down the bond.

It is interesting to note that blood and saliva contaminant groups suffered adhesive failures only (figure 4.4.) This could possibly imply an increased susceptibility within the adhesive.

The results also showed an apparent increase in the bond strength within the chlorine dioxide group when compared to the control group (p-value = 0.55 (figure 4.2, table 4.3)) although it was statistically insignificant. The chlorine dioxide disinfectant solution used was at a potable concentration of 10 parts per million. This has been shown to be sufficient to remove the bacteria within our dental unit water lines (Roberts et al., 2000; Wirthlin et al., 2001; Ritter et al., 2007; Porteous et al., 2009; Patel et al., 2012; Tuladhar et al., 2012).

This study confirms the findings of Kashani (2006) and Ritter (2007) studies that chlorine dioxide disinfectant solution had a statistically insignificant difference on dentine bond strength (Roberts et al., 2000; Kashani et al., 2006; Ritter et al., 2007) and showed a majority cohesive fracture at the dentine surface (Nassoohi et al., 2015) (table 4.3 and figure 4.4). Although insignificant the mean and median bond strength are greater than the control group. The reason for this finding is unclear but the role of reducing bacterial contamination affecting bond strength cannot be excluded.

It was striking to observe the wide range of bond strength within the control group. It is possible that this could be due to the age of the teeth with variation in intertubular dentine (Zaslansky et al., 2010, Erfan et al., 2014), which is recognised as a limitation of the study.
Limitations

We recognise the following limitations in this study:

- Age of the dentine was not known as the teeth were sampled from an extraction clinic and this data was not recorded.

- Tooth selection and sample measurement was strictly randomized. However, in order to maintain procedural accuracy and consistency the contamination materials and methods as described could not be randomized.

- This study did not subject the bonded samples to thermocycling. While previous studies, Gale et al (1999) used a standard regimen of 500 cycles in water at 5°C and 55°C. However, De Munck et al (2005a), stated that thermocycling does not affect the bond strength of the adhesive material.
CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

The manufacturers of the latest generation of bonding agents (such as Scotchbond Universal by 3M) claimed that composite bonding to dentine would be unaffected by contaminants on the dentine surface. This study however clearly showed that there was a significant reduction in the bond strength when surfaces were contaminated with blood. The null hypothesis was thus rejected.

Another interesting finding although not statistically significant, include the effects of saliva and chlorine dioxide. Within the limitations of this study, saliva did not affect the bond strength of nanocomposite to dentine, somewhat supporting the manufacturers’ claims. An unexpected finding was that chlorine dioxide showed an average greater bond than the control group. These results may warrant further investigation.

**Recommendations**

The study confirmed that blood contaminants had weakened the shear bond strength of composite to tooth dentine, maintaining the pertinent use of a rubberdam to minimise such failures. Furthermore it is recommended that this study be repeated with a combination of saliva and blood as joint contaminants. This study confirms that chlorine dioxide had no deleterious effect on the bond strength onto dentine. Chlorine dioxide solution is therefore recommended to be implemented in the dental unit waterlines as well as a dentine surface disinfectant and rinse.
REFERENCES


Csilla Csikany DDS, Varnai G, Noszticzius Z. SOLUMIUM DENTAL: the hyper-pure chlorine dioxide solution and its applications in dentistry I. 2009. Web link:


Accessed June 2015 and 2016


Dejak B, Młotkowski A. A comparison of stresses in molar teeth restored with inlays and direct restorations, including polymerization shrinkage of composite resin and tooth loading during mastication. Dent. Mater. 2015; 31:e77-87.


Hill EE, Rubel BS. Do dental educators need to improve their approach to teaching rubber dam use?. J Dent Educ. 2008;72:1177-1181


3M ESPE Filtek™ Supreme XTE Universal Restorative, 2010 booklet

Web link:
http://solutions.3m.co.za/3MContentRetrievalAPI/BlobServlet?lmd=1304690493000&locale=en_GB&assetType=MMM_Image&assetId=1273683395805&blobAttribute=ImageFile


Rweyendela IH, Patel M, Owen CP. Disinfection of irreversible hydrocolloid impression material with chlorinated compounds. SADJ. 2009; 64:208,210-212.


Suzuki TY, Gomes-Filho JE, Gallego J, Pavan S, Dos Santos PH, Briso AL. Mechanical properties of components of the bonding interface in different regions of radicular dentin surfaces. J Prosthet Dent. 2015;113:54-61


APPENDIX A

THE EFFECTS OF CONTAMINATION ON THE SHEAR BOND STRENGTH OF A SINGLE COMPOSITE USING A SELF-ETCHING ADHESIVE

If the pilot study and the main study were combined, enclosed below would be the results.

A1. Comparison to pilot study data

The pilot study data was based on the sample size calculated and compared to the main data.
For each of the four treatments, the main and pilot study data were compared by an unpaired t-test. The differences in means, the corresponding 95% confidence intervals and the p-values for the t-tests are tabulated below.

Table A1: Comparison between the pilot study and the main study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean (pilot study)</th>
<th>Mean (main study)</th>
<th>Difference in means (main-pilot)</th>
<th>95% confidence interval for difference in means</th>
<th>p-value for H0: no difference between means</th>
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<td>Blood</td>
<td>7.8</td>
<td>5.4</td>
<td>-2.4</td>
<td>-8.4 to 3.6</td>
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<tr>
<td>Chlorine Dioxide</td>
<td>16.2</td>
<td>14.5</td>
<td>-1.7</td>
<td>-8.6 to 5.3</td>
<td>0.60</td>
</tr>
<tr>
<td>Saliva</td>
<td>10.4</td>
<td>11.6</td>
<td>1.2</td>
<td>-5.8 to 8.1</td>
<td>0.72</td>
</tr>
<tr>
<td>Control</td>
<td>11.0</td>
<td>13.2</td>
<td>2.2</td>
<td>-14.6 to 19.0</td>
<td>0.64</td>
</tr>
</tbody>
</table>
There were no significant differences, for any of the treatments, between the main and pilot study data (Table A1). This validate that even though the experiments were done in a different environment and settings, there were no differences between the combined data.

**A2. Combined data**

The overall ANOVA was significant: F (3, 44) = 6.81; p=0.0007. P value is still < 0.05 indicating there were differences noticed. Post-hoc tests using Dunnett’s procedure to compare each of the three treatments to the Control treatment, showed that the mean bond strength for Blood (6.0±2.6 MPa) was significantly lower than those for Control (12.7±4.2 MPa) (values after the ± denote the 95% confidence interval for the mean). The effect size was large (Cohen’s d=1.3).

![Mean bond strength (MPa)](image)

Fig.A2. The treatment means and their 95% confidence intervals (as error bars).

In Fig. A2 had shown a comparatively similar result with chlorine dioxide disinfectant more dominant when compared to the other 3 contaminants. Blood is still significantly the lowest. The association between treatment and failure location was determined by Fisher’s exact test.
A3. Combined Data - Method of Failure

Fig. A3. Percentage of teeth in each group of the combined data (Main and pilot study)

Key:

**Method of failure:**
- **BC:** Bonding agent to composite failure
- **BD:** Bonding agent to dentine failure
- **C:** Failure at the composite
- **D:** Fracture at the dentine
- **P:** Fracture at the pulp

**Type of treatment:**
- **HB:** Blood
- **Chl Di:** Chlorine Dioxide
- **Sal:** Saliva
- **Contr:** Control

Out of 48 samples from the start of this study, examined under the stereo microscopy, Blood (HB) and Saliva (Sal) had shown predominant failure at the bonding agent to the dentine (BD) when compared to the Control group (Contr). Chlorine Dioxide (Chl Di) had shown a variation of failure sites unrelated to the bonding agent to dentine and usually a fracture at the dentine (D).
APPENDIX B
ETHICS WAIVER FORM WITH REVISED TITLE APPROVED

Human Research Ethics Committee (Medical)

Research Office Secretariat: Senate House Room SH 10005, 10th floor. Tel: +27 (0)11-717-1252
Medical School Secretariat: P V Tobie Building Room 304, 3rd Floor. Tel: +27 (0)11-717-2700
Private Bag 3, Wits 2050, www.wits.ac.za
Fax: +27 (0)11-717-1285

Ref: W-CJ-140108-1 (Revised title 07/07/2014) 24/01/2014

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the
Human Research Ethics Committee (Medical).

Investigator: Dr Vishani Soni (Student no 762660).

Project title: The effects of dentine contamination on the shear bond strength of
a self-etching adhesive and a nanocomposite.

Reason: This is a laboratory study, the saliva and blood of the the investigator will
be the contaminants there are no other human participants.

[Signature]

Professor Peter Cleaton-Jones
Chair: Human Research Ethics Committee (Medical)
## APPENDIX C

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<td>2%</td>
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</table>

### PRIMARY SOURCES

1. 1%

5/2004
Publication


5. Margeas, Robert. "Be selective: create
APPENDIX D-DECLARATION

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I, Vishavi Soni (Student number: 762660), am a student registered for the degree of Master of Science in Dentistry in the academic year 2012.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: ___________________________ Date: 18/10/13