





































































































































































## 2. Shear Bond Strength

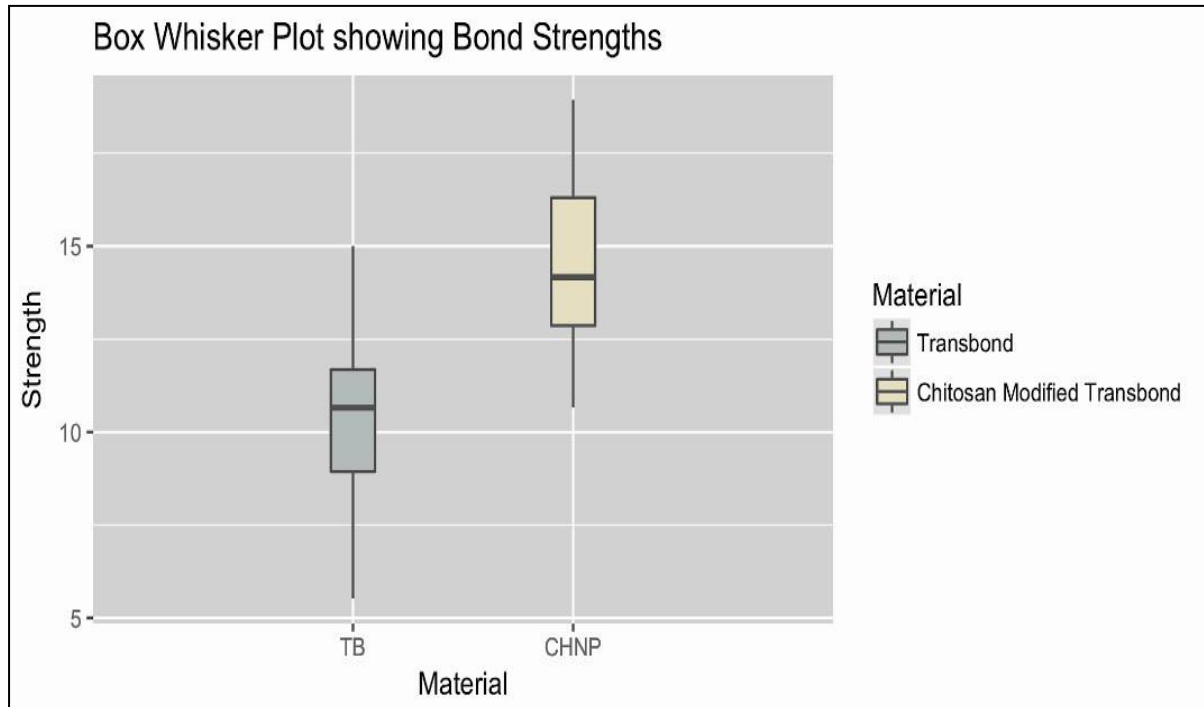


Figure 40. Box Whisker Plot showing the distribution of shear bond strength data in MPa including the median, maximum, minimum, upper and lower quartiles of the shear bond strength values for unmodified Transbond (TB) versus chitosan modified Transbond (CHNP).

Figure 40 shows the distribution of shear bond strength values obtained in the two groups. Chitosan modified Transbond (CHNP) had mean shear bond strength value of 14.5 MPa which was significantly higher than the unmodified Transbond cement with a mean shear bond strength of 10.2 MPa ( $p < 0.05$ , Wilcoxon Rank Sum Test).

The mean shear bond strength of Transbond cement showed a wider distribution of the data compared to chitosan modified cement indicating that the addition of chitosan not only produced a higher shear bond strength but also showed a narrower distribution of the data set.

When the upper half of the data sets *i.e.* the set of all values above the median values for both the test and the control samples were compared, the test samples showed higher distribution of values above the median.

### 3. Adhesive Remnant Index (ARI)

Artun and Bergland (1984), used an Adhesive Remnant Index (ARI) system to evaluate the amount of adhesive left on the tooth after de-bonding (Montasser and Drummond, 2009).

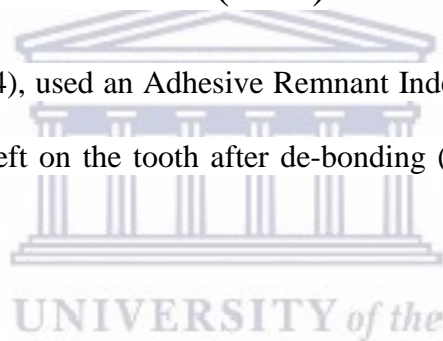


Table 1. Table showing ARI Scores 0, no adhesive on the tooth; 1, less than 50% adhesive on the tooth; 2, more than 50% adhesive on the tooth, and 3, all adhesive remained on the tooth.

	Adhesive Remnant Index Scores			
	0	1	2	3
<b>Group 1 (Control)</b>	4	14	2	-
<b>Group 2 (Test)</b>	-	18	2	-

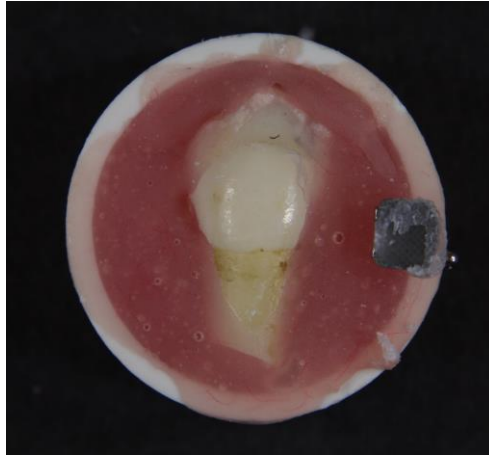


Figure 41. Picture showing the adhesive remaining on the tooth surface after bracket was broken off using the Control cement. The bracket is shown adjacent to the tooth.



Figure 42. Picture showing a bracket that was broken off, with the Control cement remaining on the bracket with an ARI score of 1.

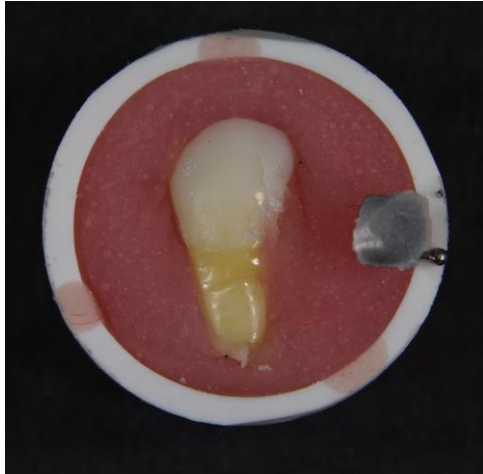


Figure 43. Picture showing the adhesive remaining on the tooth surface after bracket was broken using the Test cement. The bracket is shown adjacent to the tooth.

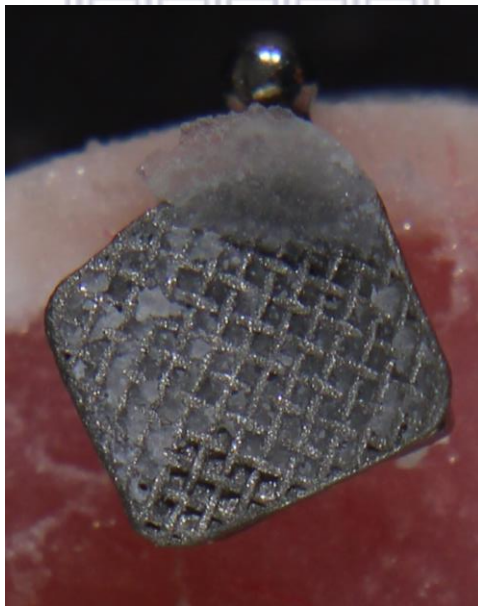


Figure 44. Picture showing a bracket broken off from the tooth using the Test cement with an ARI of 1.

The ARI scores showed only 4 specimens from the control group showed presence of cement on the bracket and nothing on the tooth surface *i.e.* a score of 0. The majority of the specimens for both the control and test group showed 50% cement remaining on the tooth surface *i.e.* a score of 1. Two specimens each from both groups showed more than 50% cement on the tooth surface *i.e.* a score of 2. There was no specimens showing 100% cement remaining on the tooth surface *i.e.* score of 3 (Table 1).

#### 4. Surface Hardness

Although the mean micro-hardness of chitosan modified cement was lower than the unmodified orthodontic cement, there was no statistically significance difference (Mann-Whitney Test,  $p > 0.05$ ). When the medians were compared, the median surface hardness of the top surface of chitosan-modified cement (Figure 45) was 47.61 (95% confidence interval for mean-upper bound 58.26; lower bound 36.95). The median surface hardness of the top surface of unmodified orthodontic cement was 43.22 (95% confidence interval for mean-upper bound 50.88; lower bound 35.56).

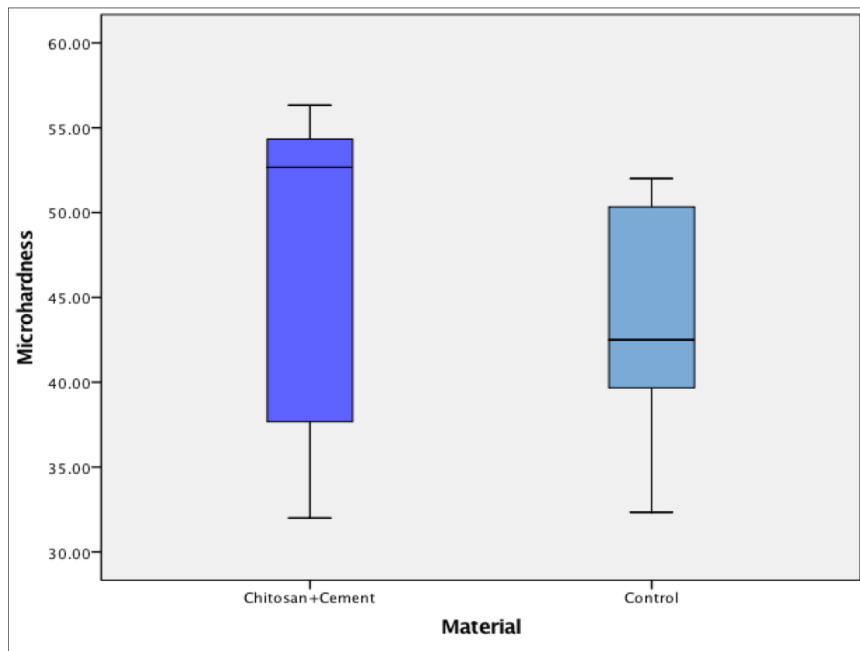
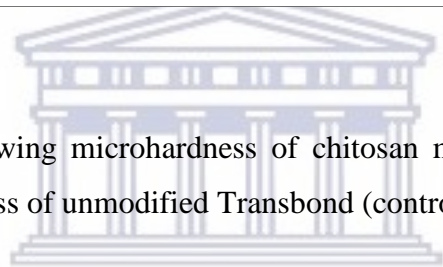


Figure 45. Box plot showing microhardness of chitosan modified Transbond cement compared to microhardness of unmodified Transbond (control).



UNIVERSITY of the  
WESTERN CAPE

## 5. Cytotoxicity

When the mouse fibroblast cells were exposed to the test sample *i.e.* the chitosan modified Transbond (Figure 46), there was an increase in the cells numbers (114.8%) compared to the controls.

Bar Chart showing Cell Viability

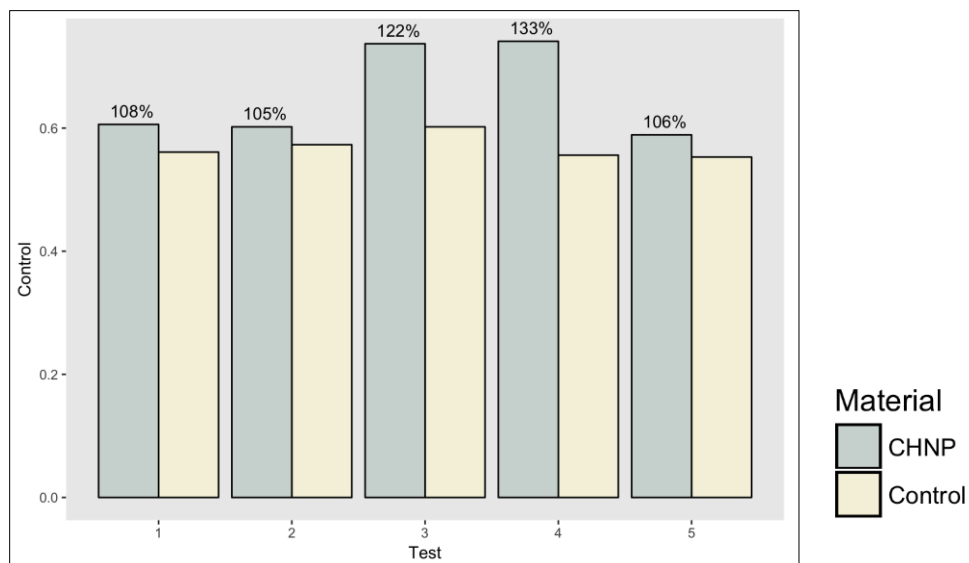


Figure 46. Graph showing increase growth of cells when cultured were exposed to Test (CHNP) compared to Control (unmodified Transbond cement).

## 6. Scanning electron microscopy analysis

Chitosan nanoparticles were observed under scanning electron microscopy at 1000, 5000 and 10 000 times magnification (Figures 47, 48, 49). Particles appear to be spherical in shape with almost evenly distributed depressions on the surface of the particles.

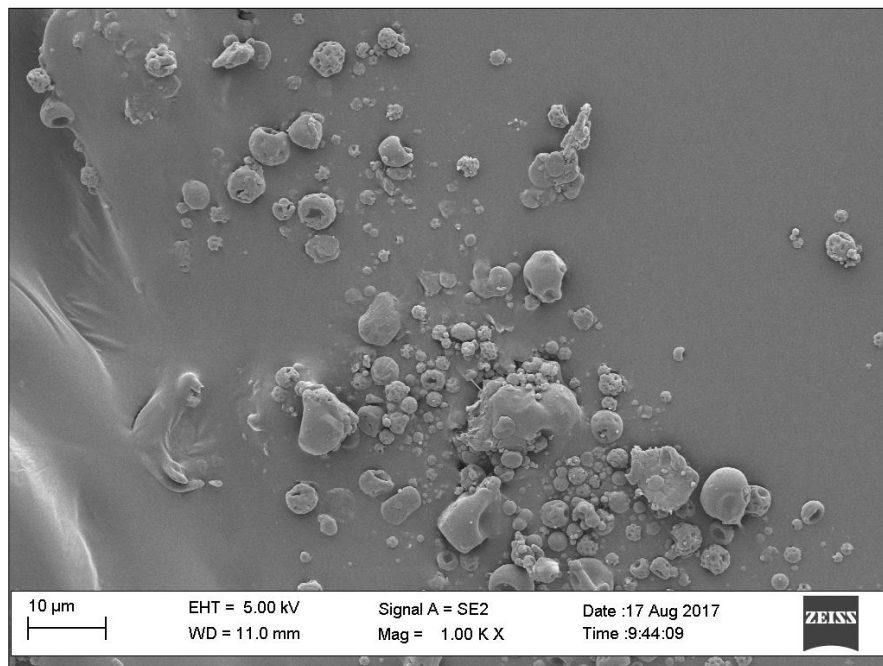


Figure 47. Scanning electron image of chitosan at 1000 magnification.

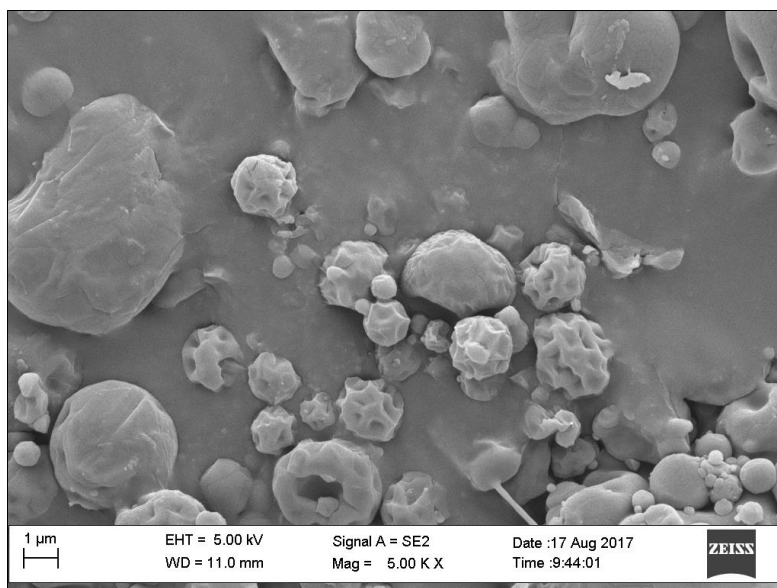


Figure 48. Scanning electron image of chitosan at 5000 magnification

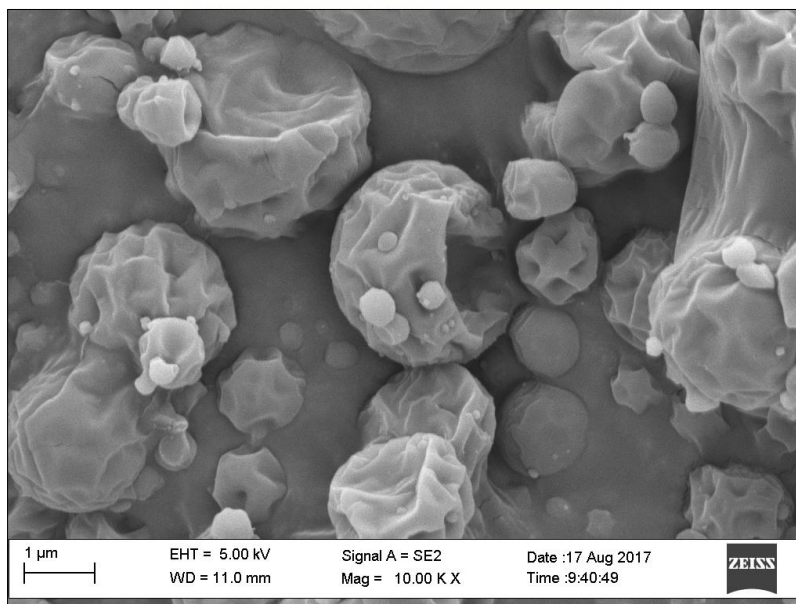
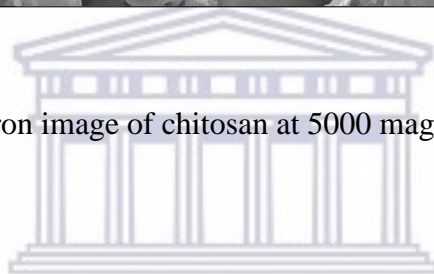


Figure 49. Scanning electron image of chitosan at 10000 magnification

# CHAPTER V

## Discussion

The evolution of Dentistry, is to a large extent, closely linked to advancements in dental materials. The introduction of dentine bonding agents saw dentistry catapult from GV Black's radical approach to cavity design to a more conservative, minimally invasive approach. Most of these so called synthetic materials are relatively inert materials. However, recently, there is an increased interest in studying the interactions between dental materials and tooth tissues that can promote bioactivity. Recently, with the introduction of bioactive materials there has been another shift in dental material science. Dental material science is in the midst of a major transition in terms of refocusing and embracing new and exciting biological technologies and might be viewed as the death of conventional dental materials (Bayne, 2005). This new shift in emphases from traditional synthetic materials toward options that involve truly biologically active materials is a field of much research in dental material science.

In 1969, Hench introduced the concept of bioactivity as "a bioactive material is one that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material" (Asthana and Bhargava, 2014). Biomaterial is therefore, any matter, surface or construct that interacts with biological systems onto which it is placed. The ideal properties of bioactive materials should be

bactericidal and bacteriostatic, sterile, stimulate dentine formation and maintain pulp vitality (Asthana and Bhargava, 2014).

This trend of studying bioactivity and biomaterials has found its way into dentistry as well. In dentistry, thus far, much of the focus on bioactive materials have been on regenerative procedures especially in the field of endodontics with materials like MTA (Dentsply, USA) and Biodentine (Septodont, USA) aimed at regeneration of dentine, for example repair of pulpal exposures. In restorative dentistry, a modification in bioactive chemically bonded cements has been introduced in the form of a calcium aluminate glass ionomer luting cement (Ceramir, Doxa Dental, USA).

Historically, orthodontic cements are inert and mainly meant to attach brackets to either bands or tooth structure. In the early days of fixed-appliance orthodontic treatment, orthodontic brackets were welded to gold or stainless-steel bands. Many developments have occurred in the decades that followed, including many newer adhesives, newer bracket materials, faster or more efficient curing methods, self-etching primers, fluoride-releasing agents, and sealants (Gange, 2015). These newer cements and bracket showed improved bonding to tooth structure. However, these developments failed to address the one major drawback of orthodontic brackets *in vivo*, and that is, the accumulation of plaque around these brackets which can lead to demineralization of the enamel upon which the bracket is cemented onto. Therefore, the purpose of this study was to address this concern of the untoward effects of orthodontic cementation of brackets with its

resultant demineralization and white spot lesions by modifying an existing orthodontic cement to a bioactive cement.

In this study, the addition of a naturally occurring cationic biopolymer, chitosan to an existing orthodontic cement (Transbond) was aimed at converting an inert cement into a novel bioactive orthodontic cement.

Biopolymers are preferred over synthetically derived polymers in regenerative medicine and dentistry because of their structural similarities with the extracellular matrix, chemical versatility, and better biocompatibility (Dutta, 2016). The International Union of Pure and Applied Chemistry (IUPAC) defines biopolymers as “macromolecules formed by living organisms”. The four important classes of biopolymers are: (a) polysaccharides; (b) proteins (c) lipids and (d) specialty polymers (McNaught and Wilkinson, 2014). Chitosan belongs to the polysaccharide group of biopolymers. Chemically, chitosan is a polymeric biopolymer comprising of N-acetylglucosamine and glucosamine copolymer units.

These naturally formed biopolymers have major advantages: biodegradability, biocompatibility, non-toxicity, cost-effectiveness, abundance, renewability, greenness and immunogenicity (Thandapani *et al.*, 2017). Among the biopolymers, chitosan a polysaccharide, offers remarkable biological properties, which have paved the way for its applications in the pharmaceutical and biomedical fields, particularly in new drug delivery systems (Dutta *et al.*, 2012; Rani *et al.*, 2010; Thandapani *et al.*, 2017). Chitosan is safe,

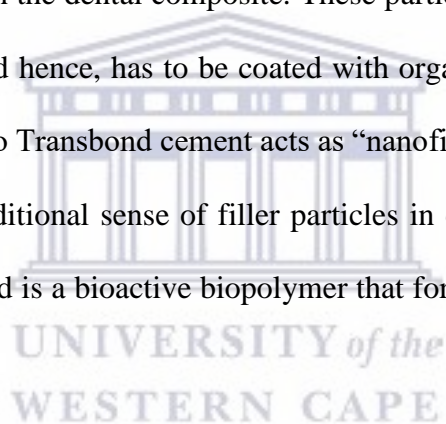
non-toxic and biocompatible polymer therefore can be used in medical and pharmaceutical applications. It is also approved by Food and Drug Administration (USA) for wound dressing (Thandapani et al. 2017).

Adding chitosan on its own to the Transbond cement will make the cement rough, as the particle size of the chitosan is fairly large. Although, the lowest molecular weight chitosan commercially available *i.e.* 50 -190 KDa was used in this study, this still presented with a challenge of large molecular size particle. Therefore, the chitosan was first converted to chitosan nanoparticles and then incorporated into the Transbond orthodontic cement. This was meant to ensure that nanoparticle size chitosan added to Transbond would not affect the surface roughness as a larger particle size may make the cement more susceptible to staining and plaque accumulation.

This utilization of nanoparticle size chitosan into the dental cement merges the fields of material science and biology. Nanoparticles hold large surface area to volume ratio which means larger surface area for higher bonding capacity. The application of larger or macro size molecules in therapy is frequently hindered by stability and/or permeation issues (Grenha, 2012). Nanoparticles have potential to easily conjugate with biomolecules and have wide application in drug delivery systems and in medicine (Rather *et al.*, 2013). Nanotechnology, nowadays is a commonly used buzzword in numerous fields of science. In the health field, the introduction of nanotechnology gave rise to a novel concept 'nanomedicine'.

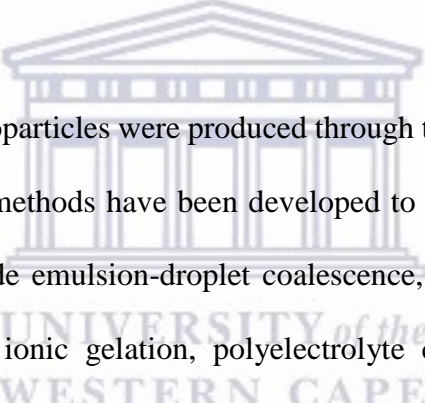
Nanotechnology is the branch of technology that deals with dimensions of nanometer size particles, especially the manipulation of individual atoms and molecules.

A variety of nanofillers have already been used in dentistry, especially in composite resin cements. 3M ESPE uses a sol-gel technology to produce tiny nanospheres which are agglomerated into nanoclusters, and either the spheres or clusters can become filler particles for composite resins (3M Technical Data, 2014). These filler particles are inert and act more to strengthen the dental composite. These particles do not chemically bond to the resin composite and hence, has to be coated with organosilanes. The inclusion of chitosan nanoparticles into Transbond cement acts as “nanofillers” in the composite resin cement but not in the traditional sense of filler particles in composites which are inert. Chitosan on the other hand is a bioactive biopolymer that forms polymer chains with the resin cement.



Chitosan nanoparticles has the advantage in that it can form a chemical network within the composite resin. Possible explanation for this is through the cationic polymerization, a type of chain growth polymerization in which a cationic initiator transfers charge to a monomer which then becomes reactive. This reactive monomer goes on to react similarly with other monomers to form a polymer. Interactions of this kind has been applied to build up polymer/polymer ionic complexes involving chitosan and negatively charged polymers.

Traditional composite resins, on the other hand, polymerize through free-radical polymerization by which polymers form by the successive addition of free-radical building blocks. Free radicals in composite resins requires the addition of separate initiator molecules like champhorquinone (Anusavice *et al.*, 2013). Owing to the cationic polymerization of the chitosan, the chitosan nanoparticles within the newly formed orthodontic cement may behave as one unit rather it simply being “inert” filler particles within the cement. Behaving a “single unit” may account for its improved physical properties in this study.



In this study, chitosan nanoparticles were produced through tripolyphosphate (TPP) ionic gelation method. Several methods have been developed to convert chitosan to chitosan nanoparticles which include emulsion-droplet coalescence, emulsion solvent diffusion, reverse micellar method, ionic gelation, polyelectrolyte complexation, spray-drying, template polymerization, precipitation and ionotropic gelation method (Grenha 2012; Thandapani *et al.*, 2017). In this study, the ionic gelation was used to produce chitosan nanoparticles and this method involves an ionic interaction between the positively charged amino groups of chitosan and the polyanion tripolyphosphate, which acts as chitosan cross-linker. Sodium tripolyphosphate (TPP) crosslinker is generally used for preparing chitosan nanoparticles. Sodium triphosphate or sodium tripolyphosphate is an inorganic compound with formula  $\text{Na}_5\text{P}_3\text{O}_{10}$  (McNaught and Wilkinson 2014). The polyphosphates are hydrolyzed into simpler phosphates, which is similar to adenosine triphosphates or

ATP, that is present within living cells (Thandapani *et al.*, 2017). Thus, the toxicity of polyphosphates is low and it has neither mutagenic or carcinogenic effects nor adverse reproductive effects (Thandapani *et al.*, 2017).

Nanoparticle formation takes place immediately after the addition of a TPP solution to a solution of chitosan (Figure 50). The production of nanoparticles by ionic gelation results in smaller particles for higher amounts of cross-linker (Grenha *et al.*, 2005; Teijeiro-Osorio *et al.*, 2009).

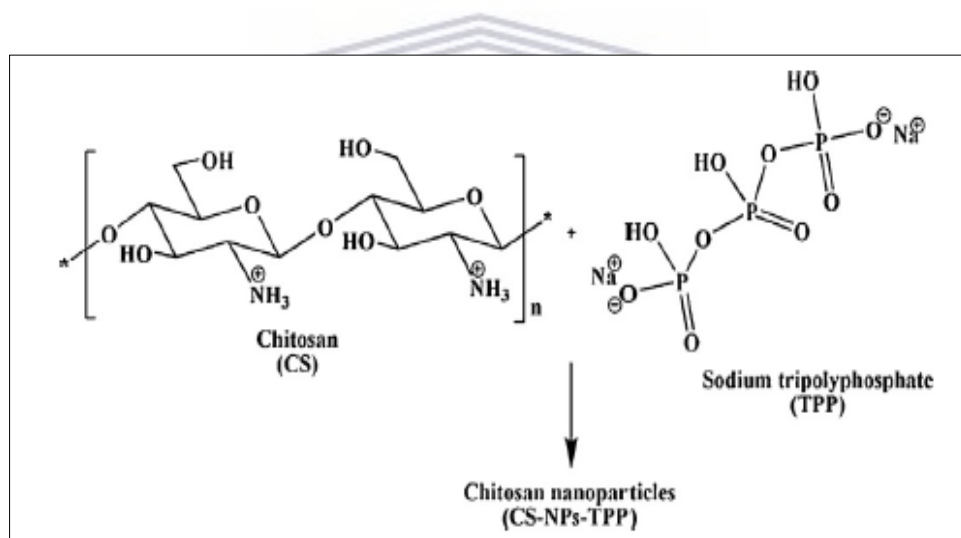


Figure 50. Preparation of chitosan nanoparticles (CS-NPs) using sodium tripolyphosphate (TPP) from chitosan (CS) (Thandapani *et al.*, 2017).

Once the chitosan nanoparticles were formed and incorporated into the cement, the question then arises, does the chitosan still maintain its biological properties within this newly formed resin cement?

## 1. *Antimicrobial effects of chitosan modified orthodontic cement*

Several bioassays for antimicrobial screening methods are available such as disc diffusion, well diffusion, broth dilution or agar dilution. Methods such as flow cytometric and bioluminescent methods are not widely used because they require specified equipment and their reproducibility and standardization still needs to be evaluated (Balouiri *et al.*, 2016). The agar disc diffusion method to determine the Minimum Inhibitory Concentration (MIC), is not appropriate as it is not possible to quantify the amount of the antimicrobial agent diffused into the agar medium (Balouiri *et al.*, 2016). In this study, because the actual antimicrobial effect of the chitosan needed to be quantified by counting the remaining colony forming units, the agar dilution method was used. The protocols as provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for this method was followed (Kahlmeter *et al.*, 2006). These guidelines provide a uniform procedure for testing that is practical to perform in most clinical microbiology laboratories. The agar dilution method is the most appropriate for the determination of minimum inhibitory concentration values, since they offer the possibility to estimate the concentration of the tested antimicrobial agent in the agar (Balouiri *et al.*, 2016). This assay does allow the bioassay to be performed in a standardized approach in order to evaluate the clinical relevance of results (Pfaller *et al.*, 2004).

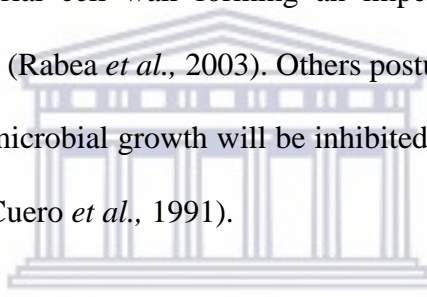
In this study, all the test samples exposed to chitosan modified Transbond showed no bacterial growth after 8 hours while the control showed an increase in bacterial growth

from baseline to 8 hours. Compared with the unmodified Transbond, the chitosan modified Transbond had higher antibacterial activity showing that the addition of chitosan to Transbond renders the cement antibacterial. Complete eradication of the bacteria was noted following 4 hours of exposure to the chitosan (Figure 31 & 32).

When the bacterial growth curves (Figure 39) were compared, the control group showed a steady increase in cell growth from baseline to final count at 8 hours. However, in the control group there was an initial drop in cell numbers after 30 minutes. This may be explained by the fact that the microorganisms needed to establish and acclimatize themselves to the new environment. The normal growth pattern was then followed after this initial adjustment period and growth occurred from 1 hour to final count at 8 hours. This steady growth in cell numbers in the control group may indicate that the Transbond cement does not possess any antibacterial properties. When the test group growth pattern was studied it showed a steady decline in cell numbers from baseline to final count at 8 hours. Complete eradication of the bacteria was noted after 4 hours of exposure to the chitosan modified cement. This decline in the cell numbers in the test group indicates that the newly formed chitosan modified Transbond orthodontic cement possesses sufficient antibacterial properties to eradicate *Streptococcus mutans* in-vitro.

Several mechanisms explaining antimicrobial activity of chitosan has been postulated. The most acceptable mechanism is the interaction between the positively charged chitosan molecules and negatively charged microbial cell membranes (Perchyonok *et al.*, 2015).

This interaction is mediated by the electrostatic forces between the protonated  $\text{NH}_3^+$  groups of chitosan and the electronegative charges of the microbial cell surfaces (Perchyonok *et al.*, 2015). Chitosan can also penetrate the bacterial cell membrane and then bind to the DNA, inhibiting its transcription and mRNA synthesis. Another alternative hypothesis for the antibacterial mechanism of chitosan is thought to be as a result of its ability to bind to the negatively charged bacterial cell membrane, increasing its permeability and ultimately resulting in leaking of the cytoplasmic contents and bacterial cell death (Qi and Xu, 2004). Chitosan binds to the negatively charged components of the bacterial cell wall forming an impermeable layer and blocking transportation into the cell (Rabea *et al.*, 2003). Others postulated that as chitosan has the ability to chelate metals, microbial growth will be inhibited by reducing enzyme activity through metal chelation (Cuero *et al.*, 1991).



## 2. *Shear bond strength of chitosan modified orthodontic cement*

Higher adhesiveness of orthodontic cements is desirable to maintain an intimate contact between orthodontic brackets and enamel thereby avoiding debonding. The purpose of the shear bond strength testing in this study was to determine whether the addition of chitosan to the bonding cement would affect the bond strength of the bracket to the enamel tooth structure.

Transbond XT composite cement was specifically developed for bonding orthodontic brackets to the enamel. According to the manufacturer (3M ESPE), the main advantages offered by this material are: reduced working time, no need for mixing, and good

adhesion to enamel thus being largely used in clinical orthodontics and experimental studies as controls and hence used in this study. When Transbond XT was used alone it showed good bond strength to enamel (10.5 MPa), which is similar to other studies done on Transbond XT (Bishara *et al.*, 2004). When Transbond XT was modified with chitosan in this study the shear bond strength increased further. Thus, the addition of chitosan to the Transbond cement improved its adhesive properties to tooth structure in this study. This improvement in adhesive properties may be explained possibly as a result of the intrinsic bioadhesive property of chitosan (Perchyonok *et al.*, 2015). The term “bioadhesion” refers to any bond formed between two biological surfaces or a bond between a biological and a synthetic surface (Perchyonok *et al.*, 2015). The water absorption capacity together with the cationic nature of chitosan which promotes binding to subsurface may be responsible for the higher bond strengths obtained when chitosan was used with Transbond cement in this study. According to Cafaggi *et al.*, (2007), hydration of the polymer causes mobilization of the polymer chains which influences polymeric adhesion (Alaçam *et al.*, 2000). Appropriate swelling is important for increase in adhesivity. However, over hydration can negatively impact on the adhesive property (Battino *et al.*, 2002). Hence, in this study the bonded teeth were stored in distilled water for 24 hours prior to testing to ensure that sufficient hydration took place prior to testing and that there was no negative effect of this hydration as this may more closely mimic the oral environment. A further advantage of addition of chitosan lies in the molecular arrangement of the polymeric chains that can interact further with the substrate (Perchyonok *et al.*, 2015) increasing adhesion to the substrate. Due to the presence of

amine groups in its structure, chitosan is converted to a polyelectrolyte in acidic media. Since many minerals and cells carry negative charges, the positive charge of chitosan interacts strongly with these negatives surfaces (Kaş, 1997).

Chitosan can bind to many materials such as cholesterol, fats, proteins and tumor cells. It has also shown an affinity for proteins. Owing to its cationic nature, electrostatic complexes are used for encapsulation of drugs, immobilization of enzymes and as a gene carrier (Kaş, 1997). For instance, fibroblasts which exhibit a more negative charge surface when compared to keratinocytes, exhibit a higher adhesion to chitosan.

When testing shear bond strength of orthodontic brackets, the location of the contact point of the de-bonding force has a significant influence on shear bond strength measurements and bond failure pattern (Klocke and Kahl-Nieke, 2005). Hence it is important to take this parameter into consideration at the start of the study design and it is especially important when comparing results with other *in-vitro* bracket bond strength results (Klocke *et al.*, 2004).

The position of the rod of the Universal Shear Bond Strength machine relative to the bracket can be positioned in 3 different contact areas as shown in Figure 51. The contact area where the force can be applied to the bracket base can be in any of these 3 locations:

1. close to the enamel/adhesive interface
2. force applied to the ligature wire groove between bracket base and wings
3. force applied to the occlusal bracket wings

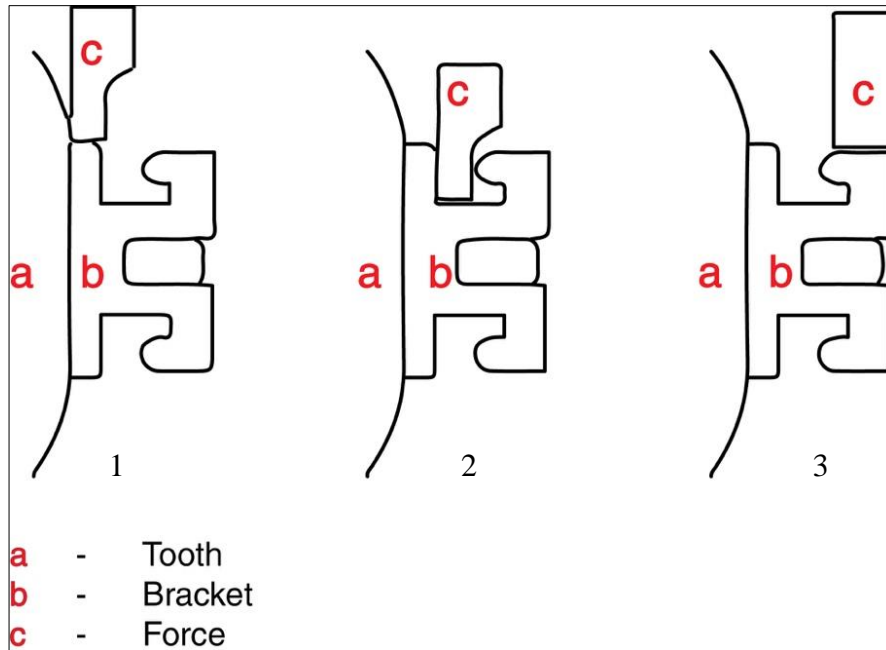


Figure 51. Diagram showing the direction of force application during de-bonding at various positions: 1. close to the enamel/adhesive interface, 2. force applied to the ligature wire groove between bracket base and wings and 3. force applied to the occlusal bracket wings. a represents the tooth surface on which the bracket b is bonded onto while c represents the position of the tip on the bracket.

In this study, the force was applied to the area close to the enamel/adhesive interface as in the clinical situation this will be where actual breakage may occur. The further the force applied from the interface, lower is the bond strength (Klocke and Kahl-Nieke, 2006). Using a similar force application point as this study, Klocke and Kahl-Nieke (2006) showed similar results to this study in which a mean shear bond strength of 11.52 MPa

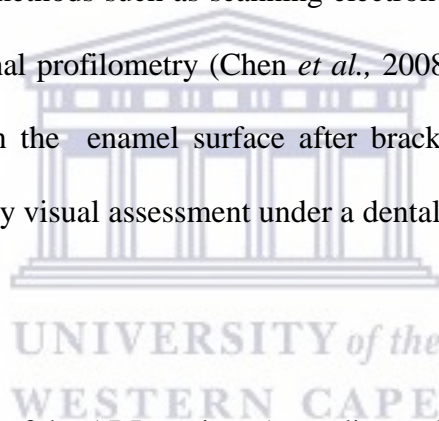
was obtained compared to 10.2 MPa obtained in this study. This study further showed that the inclusion of chitosan to the orthodontic cement improved its adhesive properties.

### *3. Adhesive remnant index (ARI)*

The term de-bonding typically suggests the removal of orthodontic brackets as well as the remaining adhesive/cement from the enamel of the tooth surface. Numerous studies in the literature give several procedures for removal of remaining adhesive and their consequences on the enamel surface (Oliver, 1988). Throughout the years, Adhesive Remnant Index or ARI scores have been one of the most commonly assessed components in research on orthodontic adhesives and quantifies the amount of remaining adhesive either on the bracket or on the tooth surface. Since the adhesive remnant score system is qualitative and subjective, various efforts have been made to adjust the original system, or to improve different quantitative approaches that can be used to more precisely evaluate the adhesive remnant (Montasser and Drummond, 2009). To more accurately assess the adhesive remnant qualitatively, several studies expanded the ARI system that was developed by Artun and Bergland (1984) including 5 or 6 scales (Uysal and Sisman, 2008). According to O'Brien *et al.* (1988), research designed to present a more accurate system for characterizing the resin remnant; this study used a quantitative method whereby a magnified image of the enamel is digitized and the amount of remaining resin is expressed as a percentage of bracket base area (Montasser and Drummond, 2009).

To precisely score the ARI is essential as it is a significant aspect to be investigated in

the variety of orthodontic adhesive (Montasser and Drummond, 2009). Precise assessment of the adhesive remnant, which is critical in the conclusive procedure of enamel clean up after de-bonding, is required for acceptable removal and restoration of the enamel surface to as close to pre-treatment condition as possible (Montasser and Drummond, 2009). Majority of laboratory analyses on the bond strength of orthodontic brackets have examined teeth and brackets under 10x magnification to evaluate and score the adhesive remnant (Uysal and Sisman, 2008), although laboratory studies aimed for assessment of the enamel surface after de-bonding and cleaning of the surface have used more sophisticated methods such as scanning electron microscope, finite element analysis, and 3-dimensional profilometry (Chen *et al.*, 2008). Clinically, evaluation of the adhesive remnants on the enamel surface after bracket de-bonding and enamel cleaning usually is done by visual assessment under a dental operating light (Montasser and Drummond, 2009).



There are many variations of the ARI scoring. According to Shamsi *et al.* (2006), the ARI scoring system is as follows:

- Score 0 = more than 75% of adhesive was left on tooth;
- Score 1 = 75% of adhesive left on tooth;
- Score 2 = 50% of adhesive left on tooth;
- Score 3 = 25% of adhesive left on tooth;
- Score 4 = less than 25% of adhesive left on tooth; and
- Score 5= no adhesive left on the tooth image.

The modified ARI was extended from the original ARI that considered adhesive remaining on the tooth surface (Artun and Bergland, 1984). The original index system (described earlier) was introduced on the basis of studies on de-bonding of extracted teeth. The ARI score based on Artun and Bergland (1984) classification was used in this study to evaluate the adhesive remaining on the tooth structure.

Even though the variations in adhesive remnant scores suggest the bonding strength, adhesive systems that show less residual resin may be preferable because they are easier to remove and safer to clean up from the enamel surface after de-bonding procedures (Oz *et al.*, 2014). As adhesive resin tags infiltrate the enamel surfaces, reaching depths up to 50  $\mu\text{m}$ , this may apply irreparable damage to the enamel surfaces once orthodontic brackets have been removed (Eminkahyagil *et al.*, 2006). Thus, an assessment system to evaluate the adhesive remnant could be helpful for researchers as well as clinicians. If adhesive remnants are not adequately detected, ARI scores could be inaccurate (Oz *et al.*, 2014).

During bracket removal, bond failure can occur at the adhesive-enamel interface or at the adhesive-bracket interface (adhesive failure), or within the adhesive (cohesive failure) (Bonetti *et al.*, 2011). Usually, bracket failure is a combination of adhesive and cohesive failures, the latter resulting in retention of material on the enamel and bracket surfaces (mixed failure). When adhesive failure amongst the adhesive resin and the enamel surface appears, a certain quantity of enamel damage is almost certain because of the

micromechanical bond between the composite resin bonding agent and the acid-etched enamel (Bonetti *et al.*, 2011). An adhesive remnant index score of 0 suggests that bond failure happened at the adhesive-enamel interface, resulting in a greater risk for tooth enamel damage (Bonetti *et al.*, 2011). In this study, 4 specimens from control group received an adhesive remnant index score of 0 where all the cement was present on the bracket. This may not be desirable as cement de-bonding may be a risk for enamel fractures. The majority of the specimens from both the control (70%) and test groups (90%) showed an adhesive remnant index score of 1 (half the amount cement remaining on the bracket), which may indicate bond failure at the bracket-adhesive interface (Bonetti *et al.*, 2011). This may be desirable as there may be minimum risk for enamel damage during de-bonding.

The ARI score system has proved to be of value in studies of orthodontic adhesive systems (Montasser and Drummond, 2009). It is a quick and simple method that needs no special equipment. However, its reliability requires investigation, with special attention on the effects of magnification on evaluation of the adhesive remnant (Montasser and Drummond, 2009). However, Delport and Grobler (1988) have questioned whether the differences in ARI scores indicate a variance in bond strength amongst the enamel and the adhesive for the different adhesive systems, but adhesive systems that display a smaller amount of adhesive remnant on the tooth has been recommended for simpler and safer removal of remaining resin after de-bonding (Guan *et al.*, 2000).

To accurately score the ARI is important because it is an important factor to be considered in the selection of orthodontic adhesive (Montasser and Drummond, 2009). Studies, (Delpont and Grobler, 1988), have debated whether the differences in ARI scores reflect a difference in bond strength between the enamel and the adhesive for the different adhesive systems, but adhesive systems that show less adhesive remnant on the tooth has been advocated for easier and safer removal of residual resin after de-bonding (Guan *et al.*, 2000). Accurate evaluation of the adhesive remnant, which is crucial in the final process of enamel cleaning after de-bonding, is needed for satisfactory removal and restoration of the enamel surface to as close to pretreatment condition as possible (Montasser and Drummond, 2009). Most laboratory studies on the bond strength of orthodontic brackets have examined teeth and brackets under 10x magnification to assess and score the adhesive remnant (Uysal and Sisman, 2008), although laboratory studies designed for evaluation of the enamel surface after de-bonding and cleaning of the surface have used more sophisticated methods such as scanning electron microscope, finite element analysis, and 3-dimensional profilometry (Chen *et al.*, 2008). Clinically, evaluation of the adhesive remnant and the enamel surface after bracket de-bonding and enamel cleanup generally is done by visual inspection under a dental operating light (Montasser and Drummond, 2009).

#### 4. *Surface hardness*

Since, surface hardness is a measure of how resistant a material is to change in shape when a compressive force is applied, it was important to determine whether the addition

of chitosan to the cement would affect its physical property. Hence, the surface hardness of the test material was determined and compared to the control using Vickers Hardness. Although the mean microhardness of chitosan modified cement was lower than the unmodified orthodontic cement, there was no statistically significant difference (Mann-Whitney Test,  $p > 0.05$ ). Thus, the addition of chitosan to Transbond did not significantly affect the surface hardness of the cement.

### *5. Biocompatibility of chitosan modified cement*

Biocompatibility refers the compatibility of manufactured materials and devices with body tissues and fluids. Biocompatibility may be defined as the ability of a material to function in a specific application in the absence of any adverse host response (Schmalz, 1994). Dental materials, as with other fields of biotechnology, need to consider compatibility of materials with tissues as one of the most important properties. A variety of *in vitro* cytotoxicity assays (also called screening tests) are available to determine the biocompatibility of materials (Freshney, 2006). In this study, guidelines as set out by The International Organization for Standardization (ISO) published the ISO 10993-5 document in 1999 to assess biological reaction to materials was used (ISO, 2009).

Cytotoxicity is the ability of a substance or material to cause damage to tissue cells. Thus, the reaction of cultured cells in cytotoxicity testing of dental materials will depend mainly on:

- 1) the material or specimen tested and/or its components and to a lesser extent to
- 2) the type of cells on which the material is tested on.

A critical variable in cytotoxic testing of dental materials is the type of cell line that is used. Currently there are differences in opinion with regards to which cell line should be used. The International Standards Organization (ISO, 2009) stated that where specific sensitivity is required, primary cell cultures, cell lines and organo-typic cultures obtained directly from living tissue can only be used if reproducibility and accuracy of the response can be demonstrated. The ISO specification does allow other cell lines to be used if the same results can be shown (ISO, 2009). Grobler *et al.* (2008) has shown similar responses to human dental pulp cells and 3T3 cell lines on various dentine bonding agents. Hence, in this study the mouse 3T3 cell lines were used.

Both the test sample *i.e.* the chitosan modified orthodontic cement and the unmodified Transbond cement were exposed to 3T3 in culture. The median cell survival rates were found to be 114.8% when the cells were exposed to chitosan modified Transbond which seem to increase the cells numbers compared to the control *i.e.* unmodified Transbond cement. The results showed that chitosan in its own capacity stimulated the cell survival rate to a value of 114.8% which is 14.8% higher than that of the control sample. Thus, it can be deduced that the presence of chitosan in the cement seems to stimulate cell growth. This may suggest that the bioactive property of chitosan is still maintained even when added to Transbond as the chitosan seems to stimulate growth of the 3T3 in culture.

## *6. Surface microstructure*

The surface microstructure was studied under scanning electron microscopy to evaluate the surface morphology of the chitosan nanoparticles. The particles were observed under 1 000, 5 000 and 10 000 times magnification and particles of varying sizes were observed. The surface morphology reflects spherical shapes structures with evenly distributed hexagonal depressions on the surface.

Thus, it seems that the chitosan presents as evenly shaped spherical particles that can be incorporated into Transbond orthodontic cement without negatively impacting on its physical properties but at the same time having a positive effect on its biological properties in that it showed antibacterial effects and a positive influence of cells in culture.

# CHAPTER VI

## Conclusion

In this study, it has been demonstrated that the newly prepared chitosan modified Transbond XT orthodontic cement possesses suitable antimicrobial activity yet at the same time also improving the adhesion to tooth structure. Thus, the addition of bioactive chitosan to Transbond presents a promising novel technology whereby the newly formed cement possesses sufficient antibacterial properties but at the same time does not compromise its bond strength.

Chitosan modified orthodontic cement demonstrated good biocompatibility by eliciting a positive growth response of 3T3 cells in culture thus demonstrating that the addition of chitosan to the orthodontic cement did not negatively affect its bioactivity.

The surface hardness between the chitosan modified cement was not negatively affected as both the unmodified cement and modified cement showed similar surface hardness.

In this study, a bioactive orthodontic cement was developed by modifying an existing orthodontic cement and evaluated for its biological and physical properties.

The science of bioactivity and nanotechnology was introduced to a dental cement to improve its antimicrobial and at the same time have superior adhesive and mechanical properties.

Further *in-vivo* studies or clinical trials will now be required to demonstrate its antimicrobial effectiveness in preventing demineralization around orthodontic brackets clinically thereby improving one of the major disadvantages of bonding brackets to tooth structure.



UNIVERSITY *of the*  
WESTERN CAPE

# CHAPTER VII

## Limitations

This is an *in-vitro* study and performed under ideal laboratory conditions where variables like conditions for microbiological growth and cell culture growth were all controlled under ideal conditions in an incubator. In the clinical situation, these conditions may vary. This study therefore, should be followed up with a randomized clinical trial.

This study was done in time intervals up to 8 hours, although complete eradication of microorganism was shown, orthodontic brackets are usually placed for prolonged periods of time. In an *in-vitro* study it is challenging to mimic the oral environment. Specimens were not aged.

This study was performed by modifying one orthodontic cement only. Further studies should be done testing chitosan on other orthodontic cements. This may then give a broader indication on how chitosan behaves with other cement formulations as well.

In this study, only *Streptococcus mutans* was used as the test bacterium. Although this may be the most commonly implicated bacterium for plaque formation, other bacteria may also be present in plaque formation. Hence, further *in-vitro* studies should investigate the effects of chitosan on other bacteria as well.



UNIVERSITY *of the*  
WESTERN CAPE

## REFERENCES

3M Technical Data. (2014). *Filtek Supreme XTE 3M*.

<http://multimedia.3m.com/mws/media/668804O/filtek-supreme-xte-flowable-restorativetechnical-data-sheet-we.pdf>. Accessed 8 November 2017

Aghoutan, H., Alami, S., Quars, F. El, Diouny, S., & Bourzgui, F. (2015). White spot lesions in orthodontic treatment and fluoride - clinical evidence. *Emerging Trends in Oral Health Sciences and Dentistry*, 317–339.

Alaçam, A., Tulunoglu, O., Oygür, T., & Bilici, S. (2000). Effects of topical Catalase application on dental pulp tissue: a histopathological evaluation. *Journal of dentistry*, 28(5), 333–339.

Anusavice, K. J., Shen, C., & Rawls, H. R. (2013). *Phillips Science of Dental Materials 12<sup>th</sup> Edition*.

Arnaud, T. M. S., De Barros Neto, B., & Diniz, F. B. (2010). Chitosan effect on dental enamel de-remineralization: An in vitro evaluation. *Journal of Dentistry*, 38(11), 848–852.

Artun, J., & Bergland, S. (1984). Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pretreatment. *American Journal of Orthodontics*, 85(4), 333–340.

Ashcraft, D. B., Staley, R. N., & Jakobsen, J. R. (1997). Fluoride release and shear bond strengths of three light-cured glass ionomer cements fluoride release for Individual Bonding Agents. *American Journal of Orthodontics and Dentofacial Orthopedics*,

111(3), 260–265.

- Aspden, T. J., Adler, J., Davis, S. S., Skaugrud, & Illum, L. (1995). Chitosan as a nasal delivery system: Evaluation of the effect of chitosan on mucociliary clearance rate in the frog palate model. *International Journal of Pharmaceutics*, 122(1–2), 69–78.
- Asthana, G., & Bhargava, S. (2014). Bioactive Materials : A Comprehensive Review. *Scholars Journal of Applied Medical Sciences (SJAMS)*, 2(6E), 3231–3237.
- Bae, K., Jun, E. J., Lee, S. M., Paik, D. I., & Kim, J. B. (2006). Effect of water-soluble reduced chitosan on Streptococcus mutans, plaque regrowth and biofilm vitality. *Clinical Oral Investigations*, 10(2), 102–107.
- Balouiri, M., Sadiki, M., & Ibsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71–79.
- Basdra, E. K., Huber, H., & Komposch, G. (1996). Fluoride released from orthodontic bonding agents alters the enamel surface and inhibits enamel demineralization in vitro. *American journal of Orthodontics and Dentofacial Orthopedics*, 109(5), 466–72.
- Battino, M., Ferreiro, M. S., Fattorini, D., & Bullon, P. (2002). In vitro antioxidant activities of mouthrinses and their components. *Journal of Clinical Periodontology*, 29(5), 462–7.
- Bayne, S. C. (2005). Dental Biomaterials: where are we and where are we going? *Journal of Dental Education*, 69(5), 571–585.
- Benson, P. (2008). Evaluation of White Spot Lesions on Teeth with Orthodontic Brackets. *Seminars in Orthodontics*, 14(3), 200–208.

- Bhattacharai, N., Gunn, J., & Zhang, M. (2010). Chitosan-based hydrogels for controlled, localized drug delivery. *Advanced Drug Delivery Reviews*, 62(1), 83–99.
- Bishara, S. E., & Ostby, A. W. (2008). White Spot Lesions: Formation, Prevention, and Treatment. *Seminars in Orthodontics*, 14(3), 174–182.
- Bishara, S., Soliman, M., & Oonsombat, C. (2004). The effect of variation in mesh-base design on the shear bond strength of orthodontic brackets. *Angle of Orthodontic*, 74(3), 400–4.
- Blackwell, J., Parker, K. D., & Rudall, K. M. (1965). Chitin in pogonophore tubes. *Journal of the Marine Biological Association of the United Kingdom*, 45(3), 659–661.
- Bloom, R. H., Brown, L. R., & City, K. (1964). A study of the effects of orthodontic appliances on the oral microbial flora. *Journal of Dental Research*, 43(5), 658–667.
- Bonetti, G. A., Zanarini, M., Incerti Parenti, S., Lattuca, M., Marchionni, S., & Rosaria Gatto Bologna, M. (2011). Evaluation of enamel surfaces after bracket debonding: An in-vivo study with scanning electron microscopy. *American Journal of Orthodontics and Dentofacial Orthopedics*, 140(5), 696–702.
- Cacciafesta, V., Sfondrini, M. F., Tagliani, P., & Klersy, C. (2007). In-vitro fluoride release rates from 9 orthodontic bonding adhesives. *American Journal of Orthodontics and Dentofacial Orthopedics*, 132(5), 656–662.
- Cafaggi, S., Russo, E., Stefani, R., Leardi, R., Caviglioli, G., Parodi, B., et al. (2007). Preparation and evaluation of nanoparticles made of chitosan or N-trimethyl chitosan and a cisplatin-alginate complex. *Journal of Controlled Release*, 121(1–2), 110–123.
- Cattani-Lorente, M. ., Godin, C., & Meyer, J. (1994). Mechanical behavior of glass

ionomer cements affected by long-term storage in water. *Dental Materials*, 10(Jan), 37–44.

Chapman, J. A., Roberts, W. E., Eckert, G. J., Kula, K. S., & Gonzalez-Cabezas, C. (2010).

Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances. *American Journal of Orthodontics and Dentofacial Orthopedics*, 138(2), 188–194.

Chen, C.-S., Hsu, M.-L., Chang, K.-D., Kuang, S.-H., Chen, P.-T., & Gung, Y.-W. (2008).

Failure Analysis: Enamel Fracture after Debonding Orthodontic Brackets. *Angle Orthodontics*, 78(6), 1071–1077.

Chien, K. B., & Shah, R. N. (2012). Novel soy protein scaffolds for tissue regeneration:

Material characterization and interaction with human mesenchymal stem cells. *Acta Biomaterialia*, 8(2), 694–703.

Chin, M. Y. H., Sandham, A., Rumachik, E. N., Ruben, J. L., & Huysmans, M. C. D. N.

J. M. (2009). Fluoride release and cariostatic potential of orthodontic adhesives with and without daily fluoride rinsing. *American Journal of Orthodontics and Dentofacial Orthopedics*, 136(4), 547–553.

Cho, Y. W., Jang, J., Park, C. R., & Ko, S. W. (2000). Preparation and solubility in acid

and water of partially deacetylated chitins. *Biomacromolecules*, 1(4), 609–614.

Chung, Y. C., Yeh, J. Y., & Tsai, C. F. (2011). Antibacterial characteristics and activity

of water-soluble chitosan derivatives prepared by the Maillard reaction. *Molecules*, 16(10), 8504–8514.

Chung, Y., & Chen, C. (2008). Antibacterial characteristics and activity of acid-soluble

- chitosan. *Bioresource Technology*, 99, 2806–2814.
- Cohen, W. J., Wiltshire, W. A., Hons, B., Dawes, C., & Lavelle, C. L. B. (2003). Containing Fluoride. *American Journal of Orthodontics and Dentofacial Orthopedics*, 124(5), 571–576.
- Coups-Smith, K. S., Rossouw, P. E., & Titley, K. C. (2003). Glass ionomer cements as luting agents for orthodontic brackets. *Angle Orthodontist*, 73(4), 436–444.
- Crisp, S., Lewis, B. G., & Wilson, A. D. (1979). Characterization cements liquid component of glass-ionomer 5 . The effect of the tartaric acid concentration. *Journal of Dentistry*, (4), 304–312.
- Croisier, F., & Jérôme, C. (2013). Chitosan-based biomaterials for tissue engineering. *European Polymer Journal*, 49, 780–792.
- Cuero, R. G., Duffus, E., Osuji, G., & Pettit, R. (1991). Aflatoxin control in preharvest maize: effects of chitosan and two microbial agents. *The Journal of Agricultural Science*, 117(2), 165.
- Delport, A., & Grobler, S. R. (1988). A laboratory evaluation of the tensile bond strength of some orthodontic bonding resins to enamel. *American Journal of Orthodontics and Dentofacial Orthopedics*, 93(2), 133–137.
- Diaz-Arnold, A. M., Holmes, D. C., Wistrom, D. W., & Swift, E. J. (1995). Short-term fluoride release/uptake of glass ionomer restoratives. *Dental Materials*, 11(2), 96–101.
- Diaz-Arnold, A., Vargas, M., & DR, H. (1999). Currebt status of luting agents for fixed prosthodontics. *Journal of Prosthetic Dentistry*, 81(2), 135–141.

- Dutta, P. K. (2016). *Chitin and Chitosan for Regenerative Medicine*. Springer.
- Dutta, P. K., Duta, J., & Tripathi, V. S. (2004). Chitin and Chitosan: Chemistry, properties and applications. *Journal of Scientific and Industrial Research*, 63(1), 20–31.
- Dutta, Tripathi, S., & Dutta, P. K. (2012). Progress in antimicrobial activities of chitin, chitosan and its oligosaccharides: a systematic study needs for food applications. *Food and Science Technology International*, 18(1), 3–34.
- Eaton, P., Fernandes, J. C., Pereira, E., Pintado, M. E., & Xavier Malcata, F. (2008). Atomic force microscopy study of the antibacterial effects of chitosans on *Escherichia coli* and *Staphylococcus aureus*. *Ultramicroscopy*, 108(10), 1128–1134.
- Eliades, T., Eliades, G., Silikas, N., & Watts, D. C. (2004). Tensile properties of orthodontic elastomeric chains. *European Journal of Orthodontics*, 26(2), 157–162.
- Eminkahyagil, N., Aman, A., Ce4tinsahin, A., & Karabulut, E. (2006). Effect of Resin-removal Methods on Enamel and Shear Bond Strength of Rebonded Brackets. *Angle Orthodontist*, 76(2), 314–321.
- Ewoldsen, N., & Demke, R. S. (2001). A review of orthodontic cements and adhesives. *American Journal of Orthodontics and Dentofacial Orthopedics*, 120(1), 45–48.
- Fajen, V. B., Duncanson, M. G. J., Nanda, R. S., Currier, G. F., & Angolkar, P. V. (1990). An in vitro evaluation of bond strength of three glass ionomer cements. *American Journal of Orthodontics and Dentofacial Orthopedics*, 97(4), 316–322.
- Featherstone, J. D. B. (2000). the Science and Practice of Caries Prevention the Science and Practice of Caries Prevention. *Journal of American Dental Association*, 131, 887–899.

- Feng, Y., & Xia, W. (2011). Preparation, characterization and antibacterial activity of water-soluble O-fumaryl-chitosan. *Carbohydrate Polymers*, 83(3), 1169–1173.
- Freshney, R. (2006). Culture of animal cells: A manual of basic technique, fifth edition. *In Vitro Cellular & Developmental Biology - Animal*, 42(5), 169.
- Fricker, J. P. (1992). A 12-month clinical evaluation of a glass polyalkenoate cement for the direct bonding of orthodontic brackets. *American Journal of Orthodontics and Dentofacial Orthopedics*, 101(4), 381–384.
- Fricker, J. P. (1994). A 12-month clinical evaluation of a light-activated glass polyalkenoate (ionomer) cement for the direct bonding of orthodontic brackets. *American Journal of Orthodontics and Dentofacial Orthopedics*, 105(5), 502–505.
- Gange, P. (2015). The evolution of bonding in orthodontics. *American Journal of Orthodontics and Dentofacial Orthopedics*, 147(4), S56–S63.
- Gaworski, M., Weinstein, M., Borislow, A. J., & Braitman, L. E. (1999). Decalcification and bond failure: A comparison of a glass ionomer and a composite resin bonding system in vivo. *American Journal of Orthodontics and Dentofacial Orthopedics*, 116(5), 518–521.
- George, M., & Abraham, T. E. (2006). Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan - a review. *Journal of Controlled Release*, 114(1), 1–14.
- Gomathysankar, S., Halim, A. S., & Yaacob, N. S. (2014). Proliferation of keratinocytes induced by adipose-derived stem cells on a chitosan scaffold and its role in wound healing, a review. *Archives of Plastic Surgery*, 41(5), 452-457.

- Gorelick, L., Geiger, A. M., & Gwinnett, A. J. (1982). Incidence of white spot formation after bonding and banding. *American Journal of Orthodontics*, 81(2), 93–98.
- Goy, R. C., Britto, D. De, & Assis, O. B. G. (2009). A review of the antibacterial activity of chitosan, *Polímeros: Ciência e Tecnologia*, 19(3), 241–247.
- Grenha, A. (2012). Chitosan nanoparticles: a survey of preparation methods. *Journal of Drug Targeting*, 20(4), 291–300.
- Grenha, A., Seijo, B., & Remuñán-López, C. (2005). Microencapsulated chitosan nanoparticles for lung protein delivery. *European Journal of Pharmaceutical Sciences*, 25(4–5), 427–437.
- Grobler, S. R., Oliver, A., Moodley, D., & Van Wyk Kotze, T. J. (2008). Cytotoxicity of recent dentin bonding agents on mouse fibroblast cells. *Quintessence International*, 39(6), 511–6.
- Grobler, S. R., Olivier, A., Moodley, D., & van W Kotze, T. W. (2004). Cytotoxicity of two concentrations of a dentine bonding agent on mouse 3T3 and human pulp fibroblast cell-lines. *SADJ: Journal of the South African Dental Association*, 59(9), 368–370,372.
- Guan, G., Takano-Yamamoto, T., Miyamoto, M., Hattori, T., Ishikawa, K., & Suzuki, K. (2000). Shear bond strengths of orthodontic plastic brackets. *American Journal of Orthodontics and Dentofacial orthopedics*, 117(4), 438–443.
- Gwinnett, A. J., & Ceen, R. F. (1979). Plaque distribution on bonded brackets: A scanning microscope study. *American Journal of Orthodontics*, 75(6), 667–677.
- Hadler-Olsen, S., Sandvik, K., El-Agroudi, M. A., & Ogaard, B. (2012). The incidence of

- caries and white spot lesions in orthodontically treated adolescents with a comprehensive caries prophylactic regimen - A prospective study. *European Journal of Orthodontics*, 34(5), 633–639.
- Hamman, J. H. (2010). Chitosan based polyelectrolyte complexes as potential carrier materials in drug delivery systems. *Marine Drugs*, 8(4), 1305–1322.
- Han, T., Nwe, N., Furuike, T., Tokura, S., & Tamura, H. (2012). Methods of N -acetylated chitosan scaffolds and its in vitro biodegradation by lysozyme. *J. Biomedical Science and Engineering*, 5(January), 15–23.
- He, P., Davis, S. S., & Illum, L. (1998). In vitro evaluation of the mucoadhesive properties of chitosan microspheres. *International Journal of Pharmaceutics*, 166(1), 75–88.
- Helbert, W., & Sugiyama, J. (1998). High-resolution electron microscopy on cellulose II and  $\alpha$ -chitin single crystals. *Cellulose*, 5, 113–122.
- Ibrahim, M. a., Neo, J., Esguerra, R. J., & Fawzy, a. S. (2015). Characterization of antibacterial and adhesion properties of chitosan-modified glass ionomer cement. *Journal of Biomaterials Applications*, 0 (0), 1–11.
- ISO. (2009a). ISO 4049:2009 - Dentistry -- Polymer-based restorative materials. <https://www.iso.org/standard/42898.html>.
- ISO. (2009b). ISO 10993-5:2009 - Biological evaluation of medical devices -- Part 5: Tests for in vitro cytotoxicity. <https://www.iso.org/standard/36406.html>.
- Jung, W.-S., Kim, H., Park, S.-Y., Cho, E.-J., & Ahn Seoul, S.-J. (2014). Quantitative analysis of changes in salivary mutans streptococci after orthodontic treatment. *American Journal of Orthodontics and Dentofacial Orthopedics*, 145(5), 603–609.

- Kahlmeter, G., Brown, D. F. J., Goldstein, F. W., MacGowan, A. P., Mouton, J. W., Odenholt, I., et al. (2006). European Committee on Antimicrobial Susceptibility Testing (EUCAST) Technical Notes on antimicrobial susceptibility testing. *Clinical Microbiology and Infection*, 12(6), 501–503.
- Kaş, H. S. (1997). Chitosan: properties, preparations and application to microparticulate systems. *Journal of Microencapsulation*, 14(6), 689–711.
- Kawashima, Y., Handa, T., Kasai, A., Takenaka, H., & Lin, S. Y. (1985). The effects of thickness and hardness of the coating film on the drug release rate of theophylline granules coated with chitosan-sodium tripolyphosphate complex. *Chemical & pharmaceutical bulletin*, 33(6), 2469–2474.
- Klocke, A., & Kahl-Nieke, B. (2005). Influence of force location in orthodontic shear bond strength testing. *Dental Materials*, 21(5), 391–396.
- Klocke, A., & Kahl-Nieke, B. (2006). Effect of debonding force direction on orthodontic shear bond strength. *American Journal of Orthodontics and Dentofacial Orthopedics*, 129(2), 261–265.
- Klocke, A., Shi, J., Vaziri, F., Kahl-Nieke, B., & Bismayer, U. (2004). Effect of time on bond strength in indirect bonding. *Angle Orthodontist*, 74(2), 245–250.
- Kong, M., Chen, X. G., Xing, K., & Park, H. J. (2010). Antimicrobial properties of chitosan and mode of action: A state of the art review. *International Journal of Food Microbiology*, 144(1), 51–63.
- Kumar, A. B. V., Varadaraj, M. C., Gowda, L. R., & Tharanathan, R. N. (2005). Characterization of chito-oligosaccharides prepared by chitosan analysis with the aid of

- papain and Pronase , and their bactericidal action against *Bacillus cereus* and *Escherichia coli*. *Biochemical Journal*, 175, 167–175.
- Kurita, K. (1998). Chemistry and application of chitin and chitosan. *Polymer Degradation and Stability*, 59(1–3), 117–120.
- Kurita, K., Tomita, K., Ishii, S., Nishimura, S. I., & Shimoda, K. (1993). B-chitin as a convenient starting material for acetolysis for efficient preparation of N-acetylchitooligosaccharides. *Journal of Polymer Science, Part A: Polymer Chemistry*, 31(9), 2393–2395.
- Le, Y., Anand, S. C., & Horrocks, A. R. (1998). Recent developments in fibres and materials for wound management, *Indian Journal of Fibre and Textile Research*, 22(4), 337–347.
- Liu, H., & Gao, C. (2009). Preparation and properties of ionically cross-linked chitosan nanoparticles. *Polymers for Advanced Technologies*, 20(7), 613–619.
- Lohbauer, U. (2010). Dental glass ionomer cements as permanent filling materials - Properties, limitations and future trends. *Materials*, 3(1), 76–96.
- López-León, T., Carvalho, E. L. S., Seijo, B., Ortega-Vinuesa, J. L., & Bastos-González, D. (2005). Physicochemical characterization of chitosan nanoparticles: Electrokinetic and stability behavior. *Journal of Colloid and Interface Science*, 283(2), 344–351.
- Markovic, E., Glišić, B., Šćepan, I., Markovi, D., & Vukoman, J. (2008). Bond strength of orthodontic adhesives. *Journal of Metallurgy*, 14(2), 79–88.
- Mattousch, T. J. H., Van Der Veen, M. H., & Zentner, A. (2007). Caries lesions after

- orthodontic treatment followed by quantitative light-induced fluorescence: A 2-year follow-up. *European Journal of Orthodontics*, 29(3), 294–298.
- McNaught, A., & Wilkinson, A. (2014). *Compendium of Chemical Terminology 2nd ed.* (A. McNaught & A. Wilkinson, Eds.) *Blackwell Scientific Publications, Oxford* (2nd ed.). Oxford.
- Melo, M. A. S., Wu, J., Weir, M. D., & Xu, H. H. K. (2014). Novel antibacterial orthodontic cement containing quaternary ammonium monomer dimethylaminododecyl methacrylate. *Journal of Dentistry*, 42(9), 1193–1201.
- Miguel, J. A. ., Almeida, M. ., & Chevitarese, O. (1995). Clinical comparison between a glass ionomer cement and a composite for direct bonding of orthodontic brackets. *American Journal of Orthodontics*, 107(5), 484–487.
- Miller, J. R. (1996). A three-year clinical trial using a glass ionomer cement for the bonding of orthodontic brackets. *Angle Orthodontist*. 66(4), 309-312.
- Millett, D. T., & McCabe, J. F. (1996). Orthodontic bonding with glass ionomer cement - a review. *European Journal of Orthodontics*, 18(4), 385–399.
- Mirani, S. Ali, Sangi, L., Kumar, N., & Ali, D. (2015). Investigating the the antibacterial effect of chitosan in dental resin composites : a pilot study. *Pakistan Oral and Dental Journal*, 35(2), 15–18.
- Mitchell, C. A., O'Hagan, E., & Walker, J. M. (1995). Probability of failure of orthodontic brackets bonded with different cementing agents. *Dental Materials*, 11(5–6), 317–322.
- Mizrahi, E. (1982). Enamel demineralization following orthodontic treatment. *American*

*Journal of Orthodontics*, 82(1), 62–67.

Montasser, M. A., & Drummond, J. L. (2009). Reliability of the Adhesive Remnant Index Score System with Different Magnifications. *Angle Orthodontist*, 79(4), 773–776.

Moreira, D. M., Oei, J., Rawls, H. R., Wagner, J., Chu, L., Li, Y., et al. (2015). A novel antimicrobial orthodontic band cement with in situ-generated silver nanoparticles. *Angle Orthodontist*, 85(2), 175–183.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1–2), 55–63.

Muzzarelli, R., Tarsi, R., Filippini, O., Giovanetti, E., Biagini, G., & Varaldo, P. E. (1990). Antimicrobial Properties of N-Carboxybutyl Chitosan, *Antimicrobial Agents and Chemotherapy*, 34(10), 2019–2023.

Ngan, P. W., Gladwin, M., Tanna, N., & Kao, E. (2009). Effects of sealant and self-etching primer on enamel decalcification. Part II: An in-vivo study. *American Journal of Orthodontics and Dentofacial Orthopedics*, 135(2), 206–213.

Nicholson, J. W., & Wilson, A. D. (2000). The effect of storage in aqueous solutions on glass-ionomer and zinc polycarboxylate dental cements. *Journal of Materials Science*, 1, 357–360.

Norevall, L. I., Marcusson, A., & Persson, M. (1996). A clinical evaluation of a glass ionomer cement as an orthodontic bonding adhesive compared with an acrylic resin. *European Journal of Orthodontics*, 18(4), 373–84.

Norris, D., McInnes-Ledoux, P., Schwaninger, B., & Weinberg, R. (1986). Retention of

- orthodontic bands with fluoride-releasing cements new. *American Journal of Orthodontics*, 89(3), 206–211.
- O'Brien, K. D., Watts, D. C., & Read, M. J. F. (1988). Residual debris and bond strength— Is there a relationship, *American Journal of Orthodontics and Dentofacial Orthopedics*, 94(3), 222–230.
- O'Reilly, M. M., & Featherstone, J. D. B. (1987). Demineralization and remineralization around orthodontic appliances: An in vivo study. *American Journal of Orthodontics and Dentofacial Orthopedics*, 92(1), 33–40.
- Ogaard, B. (1989). Prevalence of white spot lesions in 19-year-olds: A study on untreated and orthodontically treated persons 5 years after treatment. *American Journal of Orthodontics and Dentofacial Orthopedics*, 96(5), 423–427.
- Ogaard, B., Rolla, R., & Arends, J. (1988). Orthodontic appliances and enamel demineralization. *American Journal of Orthodontics and Dentofacial Orthopedics*, 94(1), 68–73.
- Oliver, R. G. (1988). The effect of different methods of bracket removal the amount of residual adhesive, *American Journal of Orthodontics and Dentofacial Orthopedics*, 93, 196–200.
- Oz, A., Yaziciog, S., Arici, N., Serdar Akdeniz, B., Murat, N., & Arıcı, S. (2014). Assessment of the Confidence of the Adhesive Remnant Index Score With Different Methods. *Turkish Journal of Orthodontics*, 26(4), 149–153.
- Pascotto, R. C., Navarro, M. F. D. L., Filho, L. C., & Cury, J. A. (2004). In vivo effect of a resin-modified glass ionomer cement on enamel demineralization around

- orthodontic brackets. *American Journal of Orthodontics and Dentofacial Orthopedics*, 125(1), 36–41.
- Perchyonok, V. T., Reher, V., Basson, N., & Grobler, S. (2015). Biomaterials for Functional Applications in the Oral Cavity via Contemporary Multidimensional Science. In V. K. Thakur & M. K. Thakur (Eds.), *Handbook of Polymers for Pharmaceutical Technologies*, (1st ed., pp. 365–412).
- Pfaller, M. A., Sheehan, D. J., & Rex, J. H. (2004, April 1). Determination of Fungicidal Activities against Yeasts and Molds: Lessons Learned from Bactericidal Testing and the Need for Standardization. *Clinical Microbiology Reviews*, 17(2), 268-280.
- Pillai, C. K. S., Paul, W., & Sharma, C. P. (2009). Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Progress in Polymer Science (Oxford)*, 34(7), 641–678.
- Qi, L., & Xu, Z. (2004). Lead sorption from aqueous solutions on chitosan nanoparticles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 251(1–3), 183–190.
- Raafat, D., Von Bargaen, K., Haas, A., & Sahl, H. G. (2008). Insights into the mode of action of chitosan as an antibacterial compound. *Applied and Environmental Microbiology*, 74(12), 3764–3773.
- Rabea, E. I., Badawy, M. E. T., Stevens, C. V., Smagghe, G., & Steurbaut, W. (2003). Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules*. American Chemical Society, 4(6), 1457-1465.
- Rani, M., Agarwal, A., & Negi, Y. S. (2010). Review: Chitosan based Hydrogel polymeric

- beads - as drug delivery system. *BioResources*, 5(4), 2765–2807.
- Rather, M. A., Sharma, R., Gupta, S., Ferosekhan, S., Ramya, V. L., & Jadhao, S. B. (2013). Chitosan-Nanoconjugated Hormone Nanoparticles for Sustained Surge of Gonadotropins and Enhanced Reproductive Output in Female Fish. *PLoS ONE*, 8(2), e57094.
- Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science*, 31(7), 603–632.
- Rinaudo, M., Pavlov, G., & Desbrières, J. (1999). Influence of acetic acid concentration on the solubilization of chitosan. *Polymer*, 40(25), 7029–7032.
- Ríos, J. L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*, 100(1–2), 80–84.
- Robertson, M. A., Kau, C. H., English, J. D., Lee, R. P., Powers, J., & Nguyen, J. T. (2011). MI Paste Plus to prevent demineralization in orthodontic patients: A prospective randomized controlled trial. *American Journal of Orthodontics and Dentofacial Orthopedics*, 140(5), 660–668.
- Rogers, S., Chadwick, B., & Treasure, E. (2010). Fluoride-containing orthodontic adhesives and decalcification in patients with fixed appliances: A systematic review. *American Journal of Orthodontics and Dentofacial Orthopedics*, 138(4).
- Rossi, F., Chatzistavrou, X., Perale, G., & Boccaccini, A. R. (2012). Synthesis and degradation of agar-carbomer based hydrogels for tissue engineering applications. *Journal of Applied Polymer Science*, 123(1), 398–408.
- Ruiz-Herrera, J., Sing, V. O., Van der Woude, W. J., & Bartnicki-Garcia, S. (1975).

- Microfibril assembly by granules of chitin synthetase. *Proceedings of the National Academy of Sciences of the United States of America*, 72(7), 2706–10.
- Sakamoto, J., Sugiyama, J., Kimura, S., Imai, T., Itoh, T., Watanabe, T., & Kobayashi, S. (2000). Artificial chitin spherulites composed of single crystalline ribbons of  $\alpha$ -chitin via enzymatic polymerization. *Macromolecules*, 33(11), 4155–4160.
- Sano, H., Shibasaki, K., Matsukubo, T., & Takaesu, Y. (2003). Effect of chitosan rinsing on reduction of dental plaque formation. *The Bulletin of Tokyo Dental College*, 44(1), 9–16.
- Schmalz, G. (1994). Use of cell cultures for toxicity testing of dental materials-advantages and limitations. *Journal of Dentistry*, 22(SUPPL. 2), S6–S11.
- Shahidi, F., Arachchi, J. K. V., & Jeon, Y. J. (1999). Food applications of chitin and chitosans. *Trends in Food Science and Technology*, 10(2), 37–51.
- Shamsi, A. Al, Cunningham, J. L., Lamey, P. J., & Lynch, E. (2006). Shear Bond Strength and Residual Adhesive after Orthodontic Bracket Debonding. *Angle Orthodontist*, 76(4), 694-699.
- Shrestha, A., Zhilong, S., Gee, N. K., & Kishen, A. (2010). Nanoparticulates for antibiofilm treatment and effect of aging on its antibacterial activity. *Journal of Endodontics*, 36(6), 1030–1035.
- Shungin, D., Olsson, A. I., & Persson, M. (2010). Orthodontic treatment-related white spot lesions: A 14-year prospective quantitative follow-up, including bonding material assessment. *American Journal of Orthodontics and Dentofacial Orthopedics*, 138(2), 136–137.

- Struszczyk, M. H. (2002). Chitin and Chitosan. *Polimery*, 47(5), 316–325.
- Sudjalim, T. R., Woods, M. G., Manton, D. J., & Reynolds, E. C. (2007). Prevention of demineralization around orthodontic brackets in vitro. *American Journal of Orthodontics and Dentofacial Orthopedics*, 131(6), 1–9.
- Summers, A., Kao, E., Gilmore, J., Gunel, E., & Ngan, P. (2004). Comparison of bond strength between a conventional resin adhesive and a resin-modified glass ionomer adhesive: An in vitro and in vivo study. *American Journal of Orthodontics and Dentofacial Orthopedics*, 126(2), 200–206.
- Surmont, P., Dermaut, L., Martens, L., & Moors, M. (1992). Comparison in shear bond strength of orthodontic brackets between five bonding systems related to different etching times : An in vitro study, *American Journal of Orthodontics and Dentofacial Orthopedics*, 414–419.
- Swartz, M. L., Phillips, R. W., & Clark, H. E. (1984). Long-term F release from glass ionomer cements. *Journal of Dental Research*, 63, 158–160.
- Tan, H., Ma, R., Lin, C., Liu, Z., & Tang, T. (2013). Quaternized chitosan as an antimicrobial agent: Antimicrobial activity, mechanism of action and biomedical applications in orthopedics. *International Journal of Molecular Sciences*, 14(1), 1854–1869.
- Teijeiro-Osorio, D., Remuñán-López, C., & Alonso, M. J. (2009). Chitosan/cyclodextrin nanoparticles can efficiently transfect the airway epithelium in vitro. *European Journal of Pharmaceutics and Biopharmaceutics*, 71(2), 257–263.
- Thandapani, G., Supriya Prasad, P., Sudha, P. N., & Sukumaran, A. (2017). Size

- optimization and in vitro biocompatibility studies of chitosan nanoparticles. *International Journal of Biological Macromolecules*, 104(B), 1794–1806.
- Tseng, H., Furuhashi, K., Ichi, & Sakamoto, M. (1995). Bromination of regenerated chitin with N-bromosuccinimide and triphenylphosphine under homogeneous conditions in lithium bromide-N,N-dimethylacetamide. *Carbohydrate Research*, 270(2), 149–161.
- Uysal, T., & Sisman, A. (2008). Can Previously Bleached Teeth Be Bonded Safely Using Self-etching Primer Systems, *Angle Orthodontist*, 78(4), 711-715.
- Van Der Merwe, S. M., Verhoef, J. C., Verheijden, J. H. M., Kotzé, A. F., & Junginger, H. E. (2004). Trimethylated chitosan as polymeric absorption enhancer for improved peroral delivery of peptide drugs. *European Journal of Pharmaceutics and Biopharmaceutics*, 58(2), 225–235.
- Verheul, R. J., Amidi, M., van Steenberghe, M. J., van Riet, E., Jiskoot, W., & Hennink, W. E. (2009). Influence of the degree of acetylation on the enzymatic degradation and in vitro biological properties of trimethylated chitosans. *Biomaterials*, 30(18), 3129–3135.
- Vorhies, A. B., Donly, K. J., Staley, R. N., & Wefel, J. S. (1998). Enamel demineralization adjacent to orthodontic brackets bonded with hybrid glass ionomer cements: an in vitro study. *American Journal of Orthodontics and Dentofacial Orthopedics*, 114(6), 668–674.
- Wang, H., Li, W., Lu, Y., Zhiliang, W., & Zhong, W. (1996). Studies on Chitosan and Poly ( acrylic acid ) Interpolymer Complex . II . Solution Behaviors of the Mixture of Water-Soluble Chitosan and Poly ( acrylic acid ). *Journal of Applied Polymer*

*Science*, 61, 2221–2224.

- Wenderoth, C. J., Weinstein, M., & Borislow, a J. (1999). Effectiveness of a fluoride-releasing sealant in reducing decalcification during orthodontic treatment. *American Journal of Orthodontics and Dentofacial Orthopedic*, 116, 629–634.
- Willmot, D. (2008). White Spot Lesions After Orthodontic Treatment. *Seminars in Orthodontics*, 14(3), 209–219.
- Wilson, A. D., Kent, B. E., Clinton, D., & Miller, R. P. (1972). The Formation and Microstructure of Dental Silicate Cements. *Journal of Materials Science Science*, 7, 220–238.
- Wisth, P. J., & Nord, A. (1977). Caries experience in orthodontically treated individuals. *Angle Orthodontist*, 47(1), 59-64.
- Yassaei, S., Davari, A., Goldani Moghadam, M., & Kamaei, A. (2014). Comparison of Shear Bond Strength of RMGI and Composite Resin for Orthodontic Bracket Bonding. *Journal of Dentistry (Tehran, Iran)*, 11(3), 282–289.
- Yi, H., Wu, L. Q., Bentley, W. E., Ghodssi, R., Rubloff, G. W., Culver, J. N., & Payne, G. F. (2005). Biofabrication with chitosan. *Biomacromolecules*, 6(6), 2881–2894.
- Younes, I., & Rinaudo, M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine Drugs*, 13(3), 1133–1174.
- Young, D. H., Köhle, H., & Kauss, H. (1982). Effect of Chitosan on Membrane Permeability of Suspension-Cultured Glycine max and Phaseolus vulgaris Cells. *Plant physiology*, 70(5), 1449–54.
- Yuan, Y., Chesnutt, B. M., Haggard, W. O., & Bumgardner, J. D. (2011). Deacetylation

of chitosan: Material characterization and in vitro evaluation via albumin adsorption and pre-osteoblastic cell cultures. *Materials*, 4(8), 1399–1416.



UNIVERSITY *of the*  
WESTERN CAPE

# APPENDIX 1



UNIVERSITY of the  
WESTERN CAPE

Oral & Dental Research Institute  
Faculty of Dentistry and WHO Oral Health Collaborating Centre  
University of the Western Cap, Cape Town

**Patient Information Sheet** to be given to the patient to take home

I, Dr Tashia Moodley am a qualified dentist involved in research and training at the University of the Western Cape, Faculty of Dentistry.

I am doing research on new dental materials and how it sticks/bonds to teeth.

After the extraction of your teeth, they are either discarded or given to the students to practice on. I wish to use your teeth to be able to determine whether a new cement I made will stick/bond to the teeth. Donating your teeth in the study is on a voluntary basis.

Donating your teeth for this study or refusing to participate will not harm or prejudice you in any way. The teeth supplied to me will not have your name on it as well as I will not be able to identify you in any way. Upon completion of this study the teeth will be discarded.

Participating in the study will definitely benefit future studies and will add to our existing pool of knowledge. All information will be kept strictly confidential.

Dr Tashia Moodley (Researcher)

I, (Patient's name)..... fully understand the information supplied to me by Dr Tashia Moodley in this information sheet

Signature: .....

Date: .....

## APPENDIX 2



UNIVERSITY of the  
WESTERN CAPE

Oral & Dental Research Institute  
Faculty of Dentistry and WHO Oral Health Collaborating Centre  
University of the Western Cape, Cape Town

### Consent form

I, Mr/Mrs/Miss.....

Date of Birth:..... File no./Hosp. Sticker.....

am willing to donate my extracted teeth in the study as described to me in the patient information letter by Dr T. Moodley. I understand that donating my teeth is voluntary.

The study is approved by the Ethical and Research Committee of the University of the Western Cape. I have been adequately informed about the objectives of the study. My rights will be protected and all my details will be kept confidential. No personal information will be published.

I hereby consent to donate my teeth for the research/study.

Patient's/patient's parent or guardian's name:.....

Patient's/patient's parent or guardian's signature:.....

Witness's name:.....

Witness's signature:.....

Researcher's signature:.....

Dr Tashia Moodley

Oral & Dental Research Institute Oral Health Centre Tygerberg

Contact details: Tel: (021) 937 3090

Mobile: 082 657 3948`

Date:.....




UNIVERSITY *of the*  
WESTERN CAPE

## APPENDIX 3

- a. Chitosan (Sigma Aldrich, USA)
- b. Glacial acetic acid (Merck, Germany)
- c. Transbond™ Orthodontic Cement (3M ESPE)
- d. Elipar™ DeepCure-S (3M ESPE)
- e. CureRite light intensity meter (Dentsply, USA)
- f. *Streptococcus mutans* (ATCC UA 159 strain, American Type Culture Collection Manassas, USA)
- g. Brain Heart Infusion (Sigma-Aldrich, St. Louis, USA)
- h. Ethylene oxide gas sterilizer (SSS; Sterile Services, Singapore).
- i. Spectrophotometer (Shimadzu, Tokyo, Japan)
- j. Phosphate Buffer Saline (Sigma-Aldrich, St. Louis, USA)
- k. Orbital Shaker Incubator (Biocom Biotech, Pretoria, RSA)
- l. Lamina Flow (Bio Flow, USA)
- m. Phosphoric acid solution (Wright Health Group Ltd, RSA)
- n. Roth stainless steel brackets (OrthoShop, RSA)
- o. Universal Testing Machine (Tinius Olsen, Horsham, USA)
- p. Vickers Hardness Machine (Zwick, Germany)
- q. Balb/c 3T3 mouse fibroblast cells ( The National Repository for Biological Materials, Sandringam, RSA)
- r. Field Emission Scanning Electron Microscope (Auriga, Zeiss, Germany)
- s. Quorum Sputter Coater (Quorum Technologies Ltd, UK)

# APPENDIX 4

The screenshot displays a Turnitin feedback studio interface. The main document area shows the following text:

  
UNIVERSITY of the  
WESTERN CAPE

Tashia Moodley

An *in-vitro* study of a modified bioactive orthodontic cement

A thesis submitted in partial fulfilment of the requirements  
for the degree of Master of Sciences (Dental)  
in the Department of Restorative Dentistry

The right sidebar, titled "Match Overview", shows a 22% match rate and a list of seven sources:

- 1 Bishara, S.E.. "White Sp..."  
Publication
- 2 www.intechopen.com  
Internet Source
- 3 Springer Series on Poly...  
Publication
- 4 www.mdpi.com  
Internet Source
- 5 www.symbiosisonlinep...  
Internet Source
- 6 nopr.niscailr.res.in  
Internet Source
- 7 mdpi.com  
Internet Source

WESTERN CAPE