surface (Noble, 2012). Grade V titanium or titanium alloy, Ti-6Al-4V, is the most commonly used material in the construction of mini-implants (Morais *et al*, 2007), with a smaller number of mini-implants being manufactured from Stainless Steel (Brown *et al*, 2014).

The risk of corrosion and release of potentially toxic ions should always be considered when making use of metals in orthodontic products. Titanium alloy contains mostly titanium but also smaller portions of aluminium and vanadium, which have been included to pure titanium to improve strength and fatigue resistance (Alves *et al*, 2016). Even though titanium is considered biologically safe and compatible with human tissues (Morais *et al*, 2007), concerns regarding aluminium and vanadium as potentially hazardous materials have been raised (Hanawa, 2004). Morais *et al* (2007) and Alves *et al* (2016) refuted these concerns. Morais *et al* (2007) showed that toxic levels of vanadium were not reached in rabbits with titanium alloys inserted into their tibiae. Alves et al (2016) performed a study where titanium alloy mini-implants were placed in artificial saliva. They did not detect any aluminium or vanadium in the artificial saliva (with the detector reading to 10µg/mL for aluminium and 0.5µg/mL for vanadium). Further analysis of the SEM images of the mini-implant surfaces showed that there was no significant corrosion. No cytotoxicity was observed, as the mini-implants immersed in artificial saliva did not affect cell viability or decrease cell metabolism. Therefore, titanium alloy is considered a biologically safe material to use in the manufacture of orthodontic mini-implants. Comparison

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of stainless steel mini-implants to titanium alloy showed that there is no difference in histological responses with these two materials (Brown *et al*, 2014).

Debate has gone around the guidelines for placement of mini-implants (Scholtz and Cook, 2009; Dorst, 2009; Cleveland and Kohn, 2009; Cope *et al*, 2009). Agreement has been made that self-drilling mini-implant placement is not a surgical procedure, whereas the pre-drilling method of mini-implant placement as well as the mini plate and palatal implant placement are considered surgical procedures (Cope *et al*, 2009; Cleveland and Kohn, 2009). Cleveland and Kohn (2009) differed only slightly in their placement technique in that they recommended usage of sterile surgical gloves in order to prevent contaminating the sterile mini-implant, whereas Cope *et al* (2009) wrote that powder-free medical exam gloves can be used because their review of the literature showed no difference in the infection rates with sterile compared to non-sterile gloves. The procedure of mini-implant placement is, therefore, well suited to be performed in the orthodontic practice.

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The use of these devices has attracted plenty of attention in contemporary orthodontics and has been a favoured topic in journals and at conferences. A recent survey (Mothobela *et al*, 2016) showed that 60.9% of South African orthodontists that responded are making use of mini-implants, with the majority having used them for at least four years. This is lower than in other countries and thought to be due to lack of manufacture of these products locally with the resulting excessive import costs and lag time. A factor that is preventing its

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propelling success is the modest, yet present failure rate associated with these mini-screws (Papageorgiou *et al*, 2012) and that these implants are occasionally required to be repositioned during the course of treatment (Chung *et al*, 2010). The cost of this treatment will be exceptionally high if a new mini-implant is to be used when one of the above occurs or is required. The most commonly reported complication (67.9%) for the South African survey (Mothobela *et al*, 2016) was failure of the mini-implants and out of those not making use of mini-implants in their practice, 22.2% stated the high cost of mini-implants was a deterring factor.

The reuse of mini-implants in the same patient will be advantageous to encourage the increase in the incorporation of these devices into orthodontic practices. Manufacturers of orthodontic mini-implants usually state that their implants are for single-use only; however, mini-implant reuse is occurring in orthodontic practice (Chung *et al*, 2010; Chung *et al*, 2014; Park *et al*, 2012). It is, therefore, important to have research displaying how cleaning and sterilising of mini-implants may alter the surface properties of the mini-implant and ultimately the host reaction on re-implantation.

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Analysis of retrieved mini-implants for the potential of re-implantation has been performed (Chung *et al*, 2014; Eliades *et al*, 2009). The main concern with re-implantation is whether the mini-implant has undergone structural changes that could result in fracture of the mini-implant on reinsertion or damage to cortical done due to increased insertion torque.

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Furthermore, remaining tissues or contamination on the retrieved mini-implants could result in microorganisms causing an osseous infection or foreign body reaction. Additional potential risks of re-implantation include bacteraemia and changes in ion release. Bacterial endocarditis associated with bacteraemia following insertion of new mini-implants has been shown, and caution should be given in patients at risk of this (Uysal *et al*, 2010).

The mechanical characteristics that these retrieved implants possess has been the main focus of most research. The retrieved mini-implants have been shown to only display deformation of the tip (Chung *et al*, 2014) and no bulk material structural changes were found when comparing to control samples (Eliades *et al*, 2009). The extent of tip deformation proportionally resulted in higher forces being required to penetrate into cortical bone with self-drilling mini-implants (Chung *et al*, 2014). These authors therefore recommended that mini-implants should not be reused due to the biomechanical and biological shortcomings of the retrieved mini-implants. However, they noted that reuse is still common practice and that if reuse is to occur then a pilot drill prior to mini-implant placement is recommended.

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Some retrieved mini-implants have also been shown to have evidence of integuments that have remained on the thread surface after removal from the patients. These integuments consist of elements such as calcium, iron deposits, carbon and phosphorus which would be expected due to the contact that mini-implant will have with biological fluids such as blood and bone particles that

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adhere to the implants (Eliades *et al*, 2009). These elements were present even after autoclave sterilisation (Chung *et al*, 2014). There are concerns regarding these surface depositions, such as immunological responses, infection, and possible inability to achieve secondary stability (Carr, 1996).

Regular methods of cleaning implants have been unsuccessful and, therefore Carr (1996) developed a method to remove all tissues that adhered to implants; in his case for orthopaedic implants. He established a three step method that made use of detergents for emulsifying lipids, followed by a dilute acid which was able to dissolve bone salts (e.g. calcium phosphate minerals) and lastly the use of sodium hypochlorite solution to remove any remaining tissues (Carr, 1996). Elements of this method were incorporated into the recently published article describing a processing method for orthodontic mini-implant reuse. This article described the processing method which includes the use of 37% phosphoric acid for 10 minutes, followed by sodium hypochlorite (5,25%) for 30 minutes (Noorollahian *et al*, 2012).

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Further interest has been shown on this topic with increasing number of studies looking into cleaning and sterilisation methods for re-implantation of mini–implants. Cleaning of mini-implants is required to help eliminate or reduce the amount of tissue remnants on the surface, which include blood, protein and other debris. Sterilisation, on the other hand, is used to “eliminate or stop reproduction of microorganisms including bacteria, spores, and fungi” (Park *et al*, 2012).

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El-Wassefy *et al* (2015) performed a study looking at autoclave, gamma radiation and ultraviolet radiation in order to sterilise mini-implants. The mini-implants that underwent the autoclave sterilisation had more positive histological results than those that underwent gamma and ultraviolet light radiation. Photomicrographs of gamma and ultraviolet light radiation-sterilised groups had signs of granulation tissue with inflammatory cells, fibroblasts proliferation and the beginning of osteoid tissue deposition, whereas autoclave-sterilised group showed signs of woven bone with irregularly arranged bone trabeculae and high cellular activity. When the mini-implants were immersed in samples of standard simulated body fluid, it was shown that the aluminium ion released at 1 day was significantly higher from the as-received, unused mini-implants compared to the retrieved, sterilised mini-implants. However, in the case of vanadium, the amount of this ion released by the unused and autoclaved groups at 1 day was significantly lower than the amount released by the mini-implants of the other sterilisation groups. After 1 month of being immersed in the simulated body fluid, the aluminium and vanadium ions in all groups had comparable significant decrease in aluminium and vanadium ion release.

A method of cleaning the mini-implants for reuse was performed by Pop Silvia *et al* (2016). They looked at using sandblasting followed by autoclave sterilisation. They showed that even though this method did not influence the maximum insertion torque, it did result in abrasive mechanical stripping of the screw surface. This could have an effect on osseointegration of the mini-implant.

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It is common for orthodontic patients to neglect to inform the orthodontic office in advance of their scheduled follow-up appointments regarding breakages. This would probably be no different in the case of failure of the mini-implant. Failure includes anything resulting in the inability of the mini-implant to act as a stationary anchor and/or necessitates it removal or replacement (Papageorgiou *et al*, 2012). Therefore, even slight mobility, which the patient may not be aware of, could be determined as failure of the mini-implant. As the practice would in most circumstances not have been made aware of the failed implant prior to the appointment, an additional appointment and delay in their treatment may occur as the currently proposed sterilisation methods in the literature would require approximately 30-45 minutes to be achieved. A method taken from a common practice in laboratory sterilisation of glass rods, which involves dipping the rod in ethanol and flaming it (Wright & Harding, 2010) may provide a solution to this problem. This may provide a quick method of sterilisation for mini-implants during the scheduled follow-up appointment that would prevent delay to the treatment. No study has looked at the use of this method to sterilise mini-implants.

There is a need to provide evidence of the quality of different sterilising methods so that a protocol for mini-implant re-implantation can be developed. This could increase the use of these devices in practice.

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CHAPTER 3: RESEARCH DESIGN AND METHODOLOGY

3.1 Study Design

The study design was a randomised controlled trial looking at three different processing methods to sterilise retrieved mini-implants for re-implantation in the same patient.

3.2 Sample Selection

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The sample consisted of retrieved mini-implants of any brand, type and length collected after use in a patient.

The retrieved sample was collected from orthodontists in the public and private sector, and a maxillo-facial and oral surgeon in the private sector in South Africa. The period of collection was between October 2015 and January 2017.

3.3 Sample Size

The retrieved sample consisted of 40 mini-implants which were each randomly allocated to one of four groups, three groups undergoing different sterilisation

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methods and one group which did not undergo any sterilisation and was considered the control group. Each group, therefore, consisted of 10 retrieved mini-implants.

3.4 Sampling Procedure

The retrieved mini-implants were placed in an Eppendorf tube immediately after removal from the patient. The Eppendorf tube that was provided to the practitioners was sterilised with a piece of filter paper and a drop of sterile water inside of the tube, and closed in a sealable bag. The practitioners closed the Eppendorf tube once mini-implant was placed in it, the bag was resealed, and the practitioner filled in the information sheet attached to the plastic bag (Addendum A). The information collected was the practitioner's name, date and time of removal of the mini-implant, date of placement of the mini-implant, patient particulars, and implant information (Brand, size, location of placement and reason for removal).

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This packet was then placed into a refrigerator (to reduce multiplication of bacteria) until collection by the investigator. In the case that the practitioner was not in the Cape Town area, a courier company was contacted and overnight delivery to Cape Town requested. The plastic bag holding the mini-implant was placed into a polystyrene box with an instant ice-pack (which was activated by the practitioner) to ensure that it remained cool during transportation.

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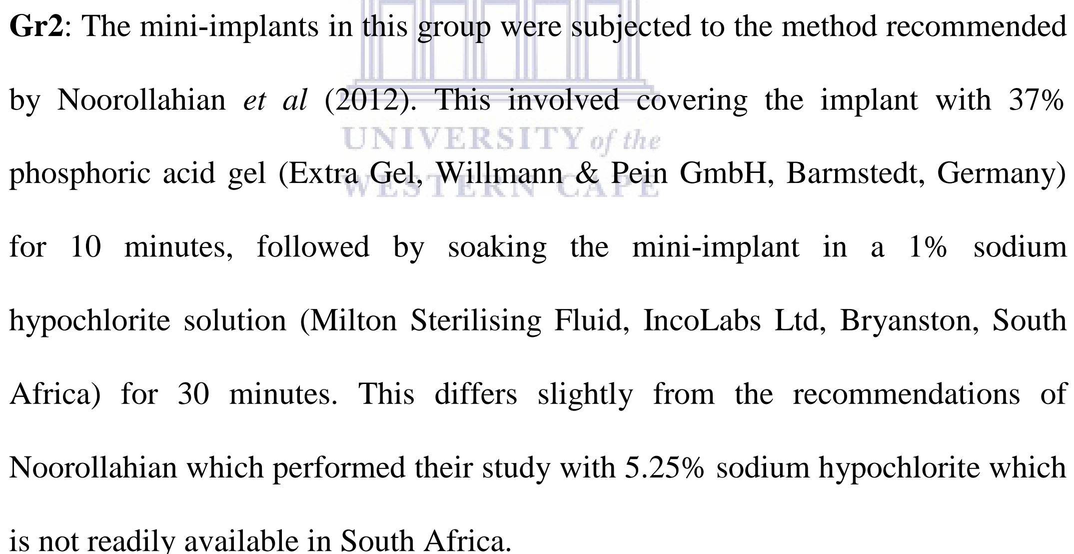
An online software programme, *Research Randomizer* (Urbaniak & Plous, 2017), was used to allocate the retrieved mini-implants to a group. Three of the groups were subjected to a different processing method and one group was not subjected to any processing method.

Gr1: The mini-implants in this group were subjected to one cycle of sterilisation in an autoclave, namely Hirayama HA-3D Autoclave (Hirayama Manufacturing Corporation, Tokyo, Japan). The autoclave was calibrated by using a biological indicator test (Attest, 3M, South Africa). Each mini-implant was placed in a separate sealable bag prior to autoclaving.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Gr2: The mini-implants in this group were subjected to the method recommended by Noorollahian *et al* (2012). This involved covering the implant with 37% phosphoric acid gel (Extra Gel, Willmann & Pein GmbH, Barmstedt, Germany) for 10 minutes, followed by soaking the mini-implant in a 1% sodium hypochlorite solution (Milton Sterilising Fluid, IncoLabs Ltd, Bryanston, South Africa) for 30 minutes. This differs slightly from the recommendations of Noorollahian which performed their study with 5.25% sodium hypochlorite which is not readily available in South Africa.

Gr3: The mini-implants in this group were processed with a method commonly used in microbiology to sterilise glass rods prior to mixing cultures. It involves dipping the mini-implant in 70% ethanol and then placing it in a Bunsen burner's flame to ignite (Wright & Harding, 2010).

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GrC: These retrieved implants did not receive any sterilisation. This was the control group.

3.5 Data Collection

Once the mini-implants were processed (if applicable), they were placed in a culture medium, namely Brain Heart Infusion medium (Oxoid Ltd, Hampshire, United Kingdom) (see Addendum B for information on this medium) which had been pre-sterilised in a test tube. This medium allowed for growth of bacteria (if present) on the mini-implant. It was placed in an incubator at 37 degrees Celsius for a minimum of 24 hours. The result of the bacterial culturing (positive or negative for bacterial growth) was read by the investigator. If the infusion medium was still clear, then a negative reading for bacterial growth was noted. If the infusion medium had a milky appearance, then a positive reading for bacterial growth was noted. The difference between the appearance of a negative and positive reading for bacterial growth is shown in Figure 3.5.1.

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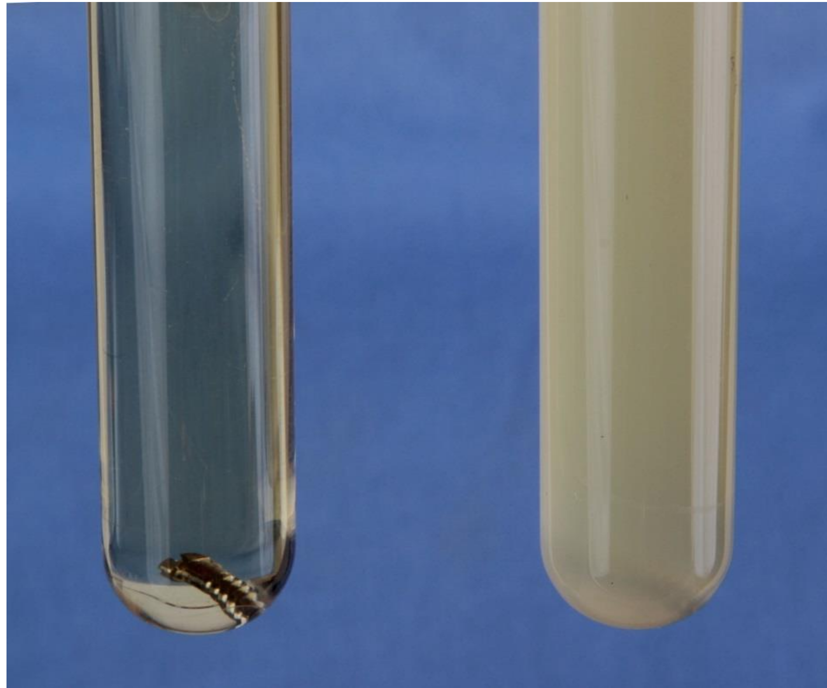

Figure 3.5.1 Photograph illustrating the difference between the appearance of a negative and positive reading for bacterial growth. Each test tube contains brain heart infusion medium and a "retrieved' mini-implant. The test tube on the left is negative for bacterial growth and the test tube on the right is positive for bacterial growth.

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After completion of this aspect of the study, the mini-implants were randomly renumbered using *Research Randomizer* to ensure blinding. The mini-implants were rinsed gently with sterile distilled water and placed in a desiccator for 48 hours.

The sample was then examined with a scanning electron microscopy to assess amount of tissue remnants remaining on their surfaces, and subjected to energy

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dispersive spectroscopy (EDS) to investigate the elemental composition of areas of the mini-implant surfaces. The scanning electron microscope with EDS that was used was the Auriga Field Emission Gun High Resolution Scanning Electron Microscope (Zeiss, Jena, Germany). This scanning electron microscope was housed at the Electron Microscopy Unit, Physics Department, University of the Western Cape, Cape Town. The imaging software that was used for the scanning electron microscope was SmartSEM (Zeiss, Jena, Germany); the images were captured at 15kV and a magnification of 27 - 43 times. The EDS software used was AZtec (Oxford Instruments, Oxfordshire, UK). The mini-implants were positioned on carbon tabs that were attached to the aluminium stubs of the holder for the Scanning Electron Microscope.

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Representative two-dimensional SEM micrographs of the mini-implants were taken for the tip, body and neck of each mini-implant. Three micrographs were, therefore, obtained for each mini-implant. These divisions are illustrated in Figure 3.5.2.

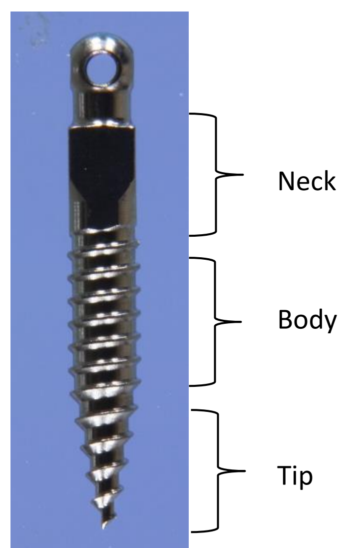

Figure 3.5.2 Photograph illustrating divisions of mini-implant

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These images, generated under the scanning electron microscope, were each placed in their own MS Word document for analysis. A table was placed over the image and the dimensions of each cell of the table were specified to 0.4cm X 0.4cm. The cells that were covering the areas of visible tissue remnants or alterations to the surface appearance on the retrieved mini-implants were highlighted in red (Figure 3.5.3). A duplicate MS Word document was created for each mini-implant image. In this document, all the cells of the table that were covering the entire mini-implant were highlighted in yellow to determine the surface area (Figure 3.5.4).

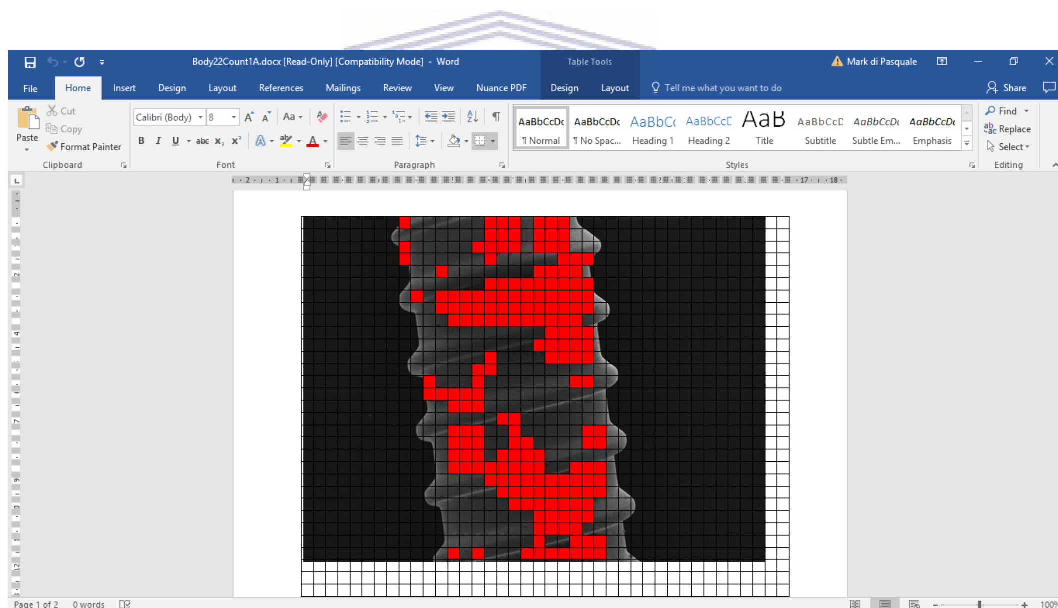

Figure 3.5.3 Screenshot displaying the process in MS Word of selecting the areas of visible tissue remnants or surface alteration on the images obtained from the scanning electron microscope analysis of the retrieved mini-implants

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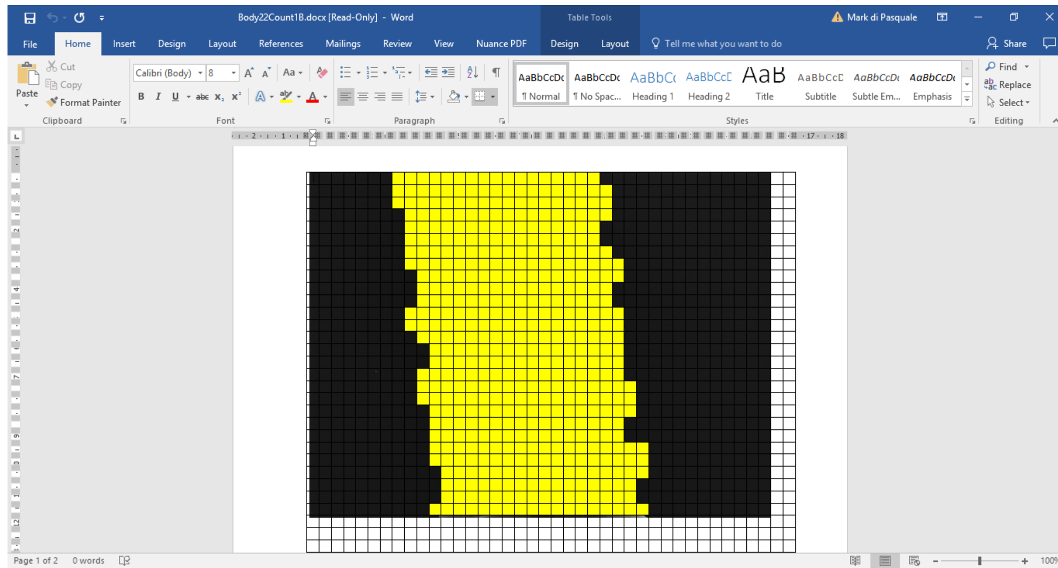
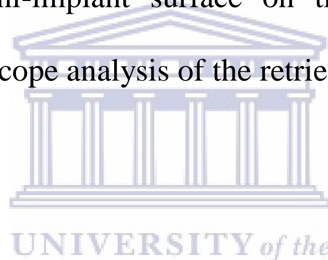


Figure 3.5.4 Screenshot displaying the process in MS Word of selecting the areas covering the entire mini-implant surface on the images obtained from the scanning electron microscope analysis of the retrieved mini-implants



These tables were then exported into a MS Excel worksheet. An Add-in Macro was developed to count the cells by colour in MS Excel. The code for this Macro was obtained from an online software development company, AbleBits, providing Add-ins for Microsoft Office (Cheusheva, 2017). A value for the number of cells in red (total tissue remnants/surface alterations) and a value for the number of cells in yellow (surface area of the mini-implant) were obtained by using this "CountCellsByColor" function (Figure 3.5.5). These values were obtained for the images of the tip, body and neck of each mini-implant; and the values were inserted into a MS Excel worksheet. All the values for the visible tissue remnants on the tip, body and neck of each mini-implant were added together and the same

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performed for the surface area. A percentage of visible tissue remnants on the surface of the mini-implant were obtained using these summed values.

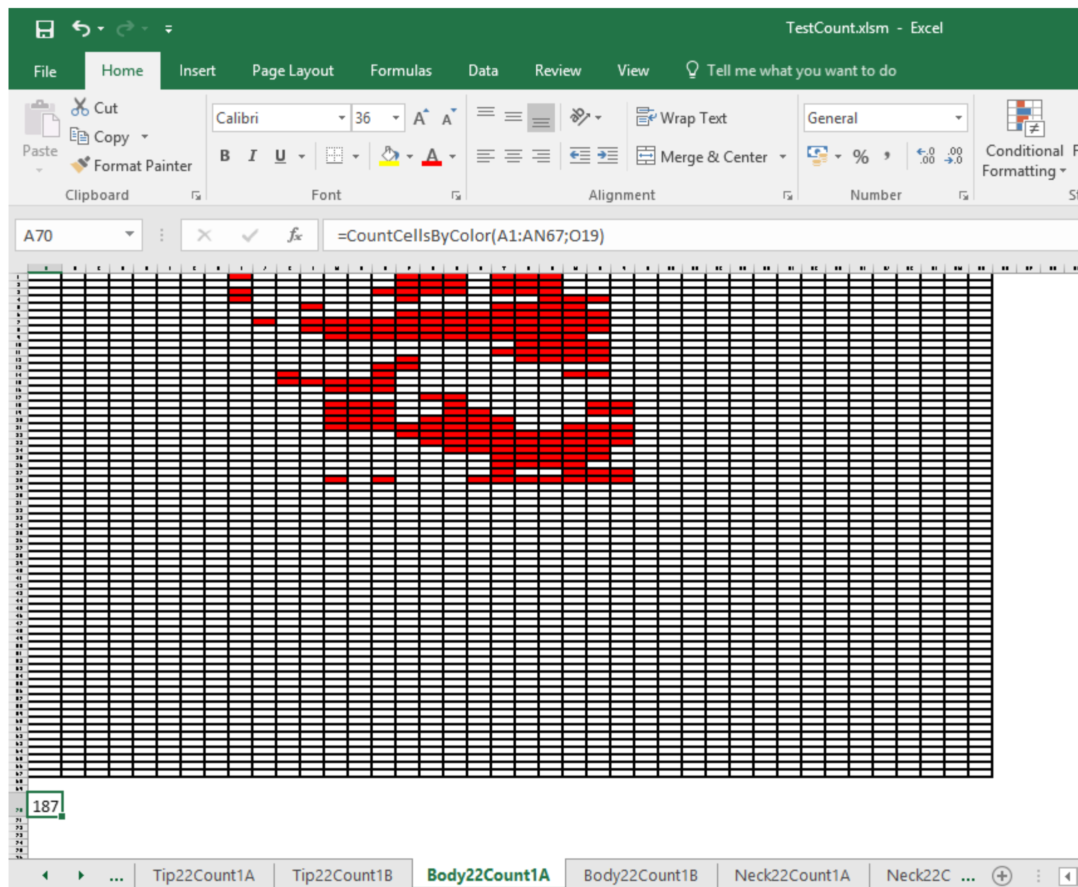

Figure 3.5.5 Screenshot displaying the use of the "CountCellsByColor" function in MS Excel

EDS was performed on randomly selected areas on the mini-implants representing visibly "clean" or "contaminated" areas. The areas defined as "contaminated" were those that displayed visible surface remnants or alterations. There were between one and five areas selected for each section of study on the mini-implant, namely the tip, body and neck. The elements found in the selected areas were

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viewed in atomic percentages. Analysis of these selected areas and the resulting elemental composition was manually examined by the investigator, and representative images selected for further discussion in this report. All the visibly “clean” and “contaminated” areas for each group of retrieved mini-implants were all also grouped together in MS Excel. Through filtering methods and pivot tables in MS Excel, the mean and standard deviation for each element in the different zones of the mini-implants were found.

3.6 Data Analysis

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Descriptive analysis was performed in MS Excel and analytical statistics was performed in statistical programme IBM SPSS Statistics Version 24 (SPSS Inc., Chicago, Ill.).

3.7 Ethical Clearance

Ethical approval was obtained to perform this research through the Senate Research Committee at the University of the Western Cape. The Project Registration Number is 15/7/29 (Addendum C).

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CHAPTER 4: RESULTS

4.1 Description of Sample

Nine practitioners from around South Africa contributed retrieved mini-implants to this study. Six were Orthodontists in private practice, two were Orthodontic registrars at the University of the Western Cape and one was a Maxillo-facial and Oral surgeon in private practice.

The retrieved mini-implants consisted of 12 (30%) ***3M Unitek*** (3M ESPE, St. Paul, USA) mini-implants, 9 (22.5%) ***Dual-Top Anchor system*** (Jeil Medical Corporation, Seoul, Korea) mini-implants, 7 (17.5%) ***BENEfit*** (PSM Medical Solutions, Tuttlingen, Germany) mini-implants, 6 (15%) ***VectorTAS*** (Ormco, Glendora, USA) mini-implants, 2 (5%) ***Infinitas*** (DB Orthodontics Limited, West Yorkshire, United Kingdom) mini-implants, 2 (5%) ***Synthes*** (Synthes, Oberdorf, Switzerland) mini-implants, 1 (2.5%) ***Aarhus System*** (American Orthodontics, Sheboygan, USA) mini-implant and 1 (2.5%) ***Tomas*** (Dentaurum, Ispringen, Germany) mini-implant. This is distribution of brands is displayed in Figure 4.1.1

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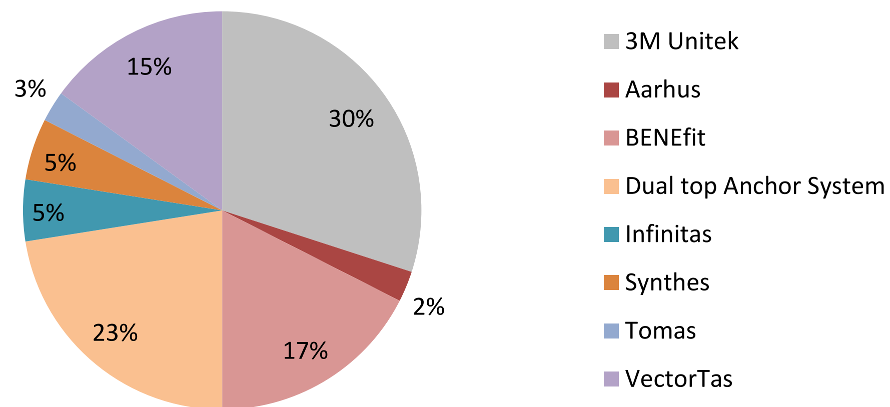

Figure 4.1.1 Distribution of Brands

Analysis of which systems were used by the practitioners showed that VectorTas was used by three practitioners, 3M Unitek and BENEfit was used by two practitioners each, and Dual top Anchor system, Aarhus System, Infinitas, Synthes and Tomas were used by one practitioner each.

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Table 4.1.1 Systems used by practitioners

	VectorTas	3M Unitek	BENEfit	Dual top	Aarhus	Infinitas	Synthes	Tomas
No. using brand	3	2	2	1	1	1	1	1

Comparison of the length of the retrieved mini-implants is shown in Figure 4.1.2. The most commonly used length of mini-implant was 6mm.

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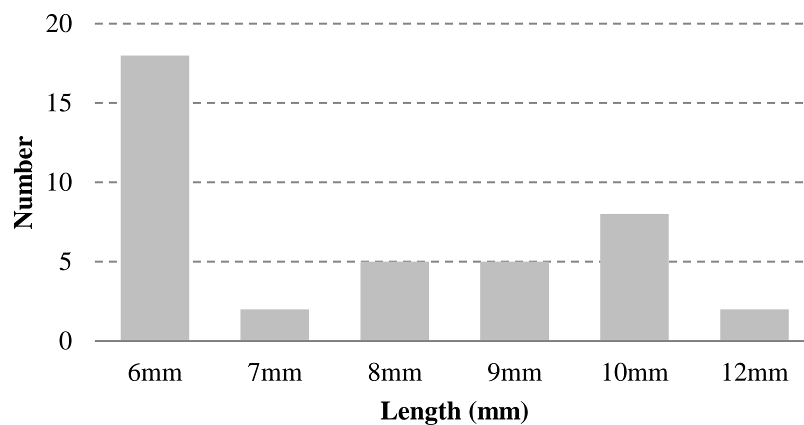

Figure 4.1.2 Distribution of Lengths

The noted areas of placement of the mini-implants were pooled into one of four groups, namely, palatal, anterior buccal, posterior buccal and mandibular buccal shelf. Most, 21 (52.5%), of the retrieved mini-implants were placed ***palatally***, 9 (22.5%) were placed in the ***posterior buccal*** area, 8 (20%) were placed in the ***anterior buccal*** area, and 2 (5%) were placed in the ***mandibular buccal shelf*** area. This is displayed in Figure 4.1.3.

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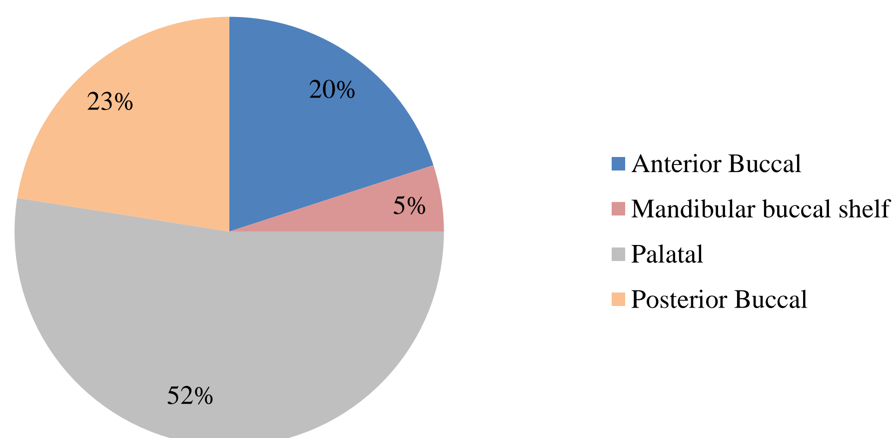

Figure 4.1.3 Location of mini-implant placement

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The reasons for removal of the mini-implants were categorised into four groups: 30 (75%) mini-implants were removed as ***treatment was completed***, 6 (15%) were removed as they were ***no longer needed***, 2 (5%) because patient ***transferred*** and 2 (5%) because mini-implant ***failed***. The failed mini-implants were placed palatal and in the mandibular buccal shelf.

Table 4.1.2 Reasons for removal of mini-implants

Reason for removal	No. of Implants
Failed	2
No longer needed	6
Transferred	2
Treatment completed	30

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The age of the patients from whom the mini-implants were retrieved ranged from 10.78 to 46.84 years with a mean of 20.48 years. The length of time the mini-implants were in the mouth also had a large range of 0.15 to 2.56 years, with an average of 1.24 years (shown in Table 4.1.3).

Table 4.1.3 Descriptive statistics for age of patients, length of use of mini-implant, and time between removal and processing

	Mean	Std. Deviation	Minimum	Maximum
Age at date of placement (Years)	20.48	9.66	10.78	46.84
Length of use of mini implant (Years)	1.24	0.57	0.15	2.56
Lead time from removal to processing (Hours)	48.84	35.19	3.50	124.58

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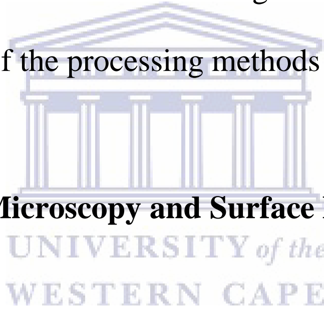
4.2 Bacterial Analysis

All the mini-implants in the groups that underwent sterilisation (Gr1, Gr2 and Gr3) were negative to bacterial growth and those in the control group (GrC) were positive to bacterial growth. The average time between removal of the mini-implant and processing was 48.84 hours. There was a large range, 3.5 to 124.58 hours, due to complications with the courier service not being available on "non-working days". However, due to the fact that the mini-implants in the groups that underwent sterilisation were all negative to bacterial growth and those in the control group were all positive to bacterial growth, this variable of time had no influence on the quality of the processing methods to achieve sterilisation.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4.3 Scanning Electron Microscopy and Surface Element Analysis

4.3.1 Representative Micrographs

A representative micrograph of the body of one of the ten mini-implants in each group was selected for display in this report. This is shown in figure 4.3.1. There are visible differences in the surface appearances between the mini-implants with regards to the remaining tissue remnants. All the retrieved mini-implants showed varying amounts of tissue remnant on their surfaces, however, those processed in with Etch and Milton (Gr2) showed minimal to no surface contaminants. Those that were autoclaved (Gr1) or burnt (Gr3) had clear tissue remnants on the

surface. Interestingly, GrC, where no sterilisation was performed, displayed less visible tissue remnants than G1 and Gr3.

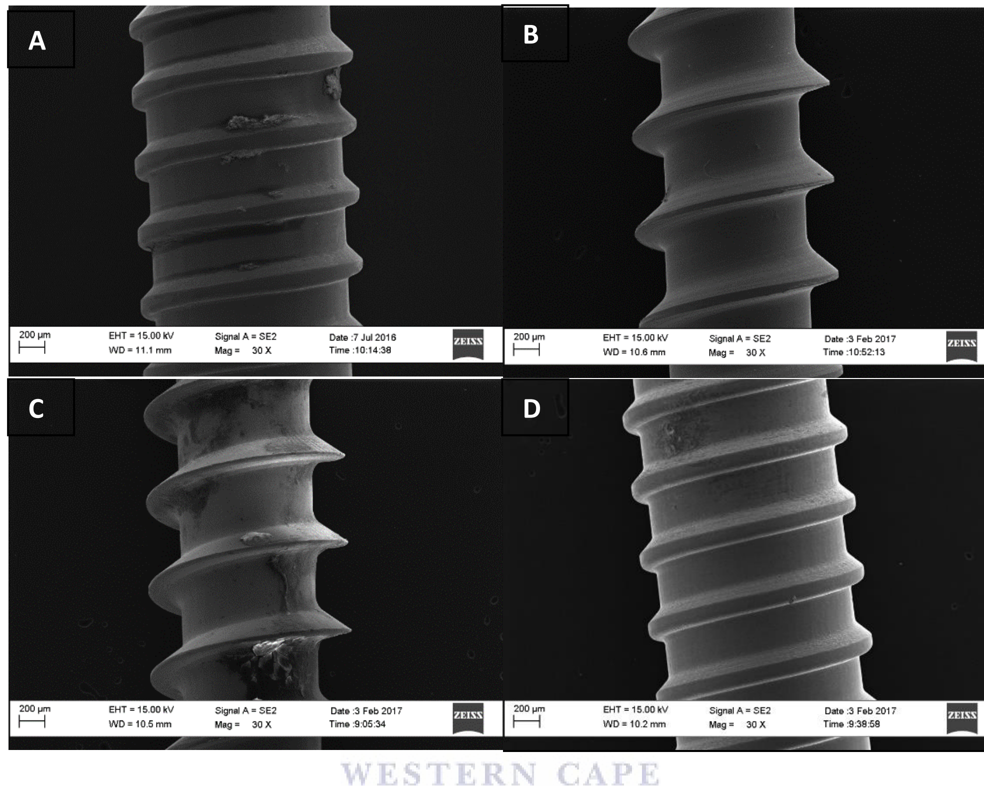

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Figure 4.3.1 Representative Scanning Electron Micrographs of the body surface of retrieved mini-implants. A. Gr1 – Autoclaved, B. Gr2 – Etch and Milton, C. Gr3 – Burnt, D. GrC - Nothing

Gr1 (Autoclave) shows areas of elevated tissue remnants, mostly along the thread of the mini-implant, as well as areas of darkening/discolouration on the smooth implant surface. From the mini-implant in the representative image 4.3.1 A, the spot element analysis of the elevated area of tissue remnants consisted of mostly carbon and oxygen with smaller amounts of calcium, sodium, phosphorus and

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titanium. The areas of discolouration in Gr1 displayed mostly carbon, oxygen and titanium with smaller percentages of aluminium, phosphorus and calcium.

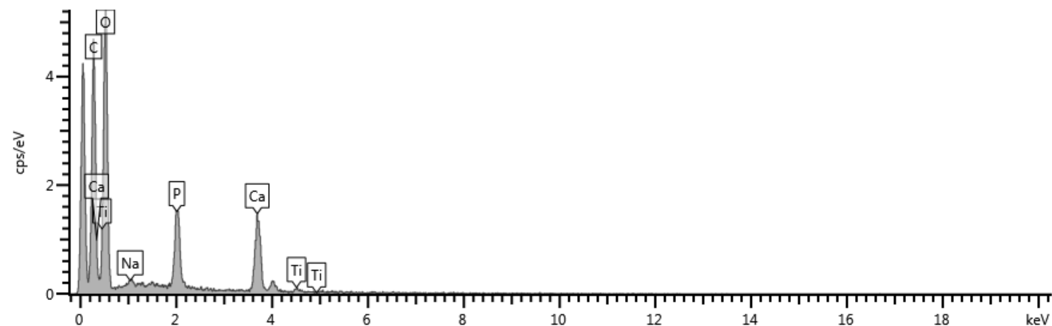

Figure 4.3.2 EDS spot analysis spectra on a region of the tissue remnant seen on Figure 4.3.1 A, Autoclave group.

Gr2, Etch and Milton, shows minimal areas of remnants or surface changes on all of the images. The remnants that are present are very small, almost pin point areas. The element spectra graph from the pin point darkened area in figure 4.3.1B is shown in Figure 4.3.3 to be titanium, aluminium, vanadium and chlorine. The arrangement of the elements is very similar to an unused mini-implant (with the addition of chlorine).

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Figure 4.3.3 EDS spot analysis spectra on a region of the tissue remnant seen on Figure 4.3.1 B, Etch and Milton group.

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Gr3, Burnt, shows larger areas of discolouration/darkening than the other groups alongside areas of elevated tissue remnants. The darkened areas were both smooth and elevated. The areas of calcified tissue remnants were not isolated to the threads and were found equally on the tip, body and neck of the mini-implants. A spot analysis of the elements in the tissue remnants showed mostly carbon and oxygen, but included sodium, titanium, aluminium, phosphorus, sulphur, potassium and calcium.

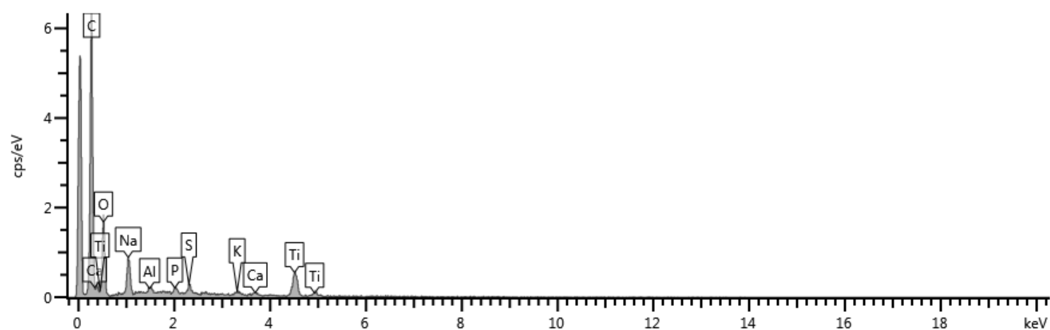

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Figure 4.3.4 EDS spot analysis spectra on a region of the tissue remnant seen on Figure 4.3.1 C, Burnt group.

GrC showed slightly more tissue remnants that Gr2, yet still minimal areas of tissue remnants including elevated areas and darkened areas. A spot analysis of the tissue remnants showed in Figure 4.3.1 D shows mostly oxygen, carbon, calcium, phosphorus, and smaller amounts of sodium, titanium, aluminium and manganese.

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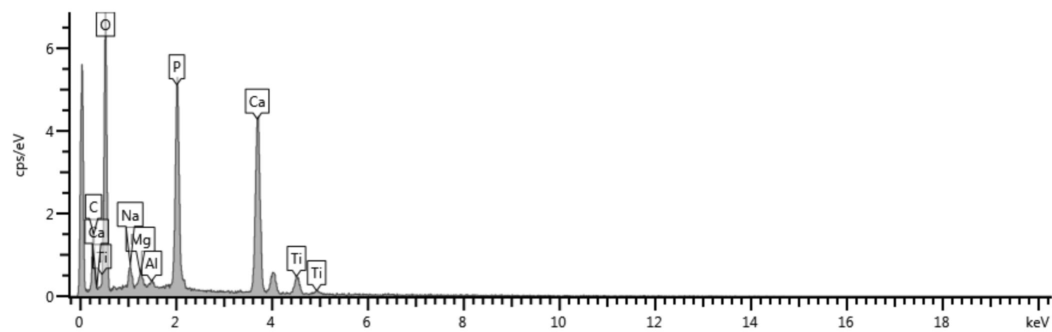

Figure 4.3.5 EDS spot analysis spectra on a region of the tissue remnant seen on Figure 4.3.1 D, Control group.

4.3.2 Comparison of Surface Element Analysis

The results of the element analysis of areas with and without visible tissue remnants for each group were pooled together for comparison and displayed in tables 4.3.1 to 4.3.4.

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Table 4.3.1 Atomic percentage of elements in areas with visible tissue remnants

	Areas with Visible Tissue Remnants					
	Body		Neck		Tip	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
C						
Autoclave	55.93	19.90	52.53	23.74	52.82	20.57
Burnt	54.26	23.91	59.89	17.35	45.09	24.12
Etch and Milton	28.54	27.37	26.97	30.87	35.63	30.06
Nothing	31.81	25.39	38.47	28.73	38.20	31.06
O						
Autoclave	26.80	12.90	26.10	14.69	25.63	16.31
Burnt	23.79	12.46	23.89	7.15	18.40	15.31
Etch and Milton	10.37	12.56	22.95	20.39	18.72	18.64
Nothing	18.37	20.04	26.09	22.23	23.82	20.37
Ti						
Autoclave	6.53	12.49	15.36	25.44	11.95	15.75
Burnt	14.59	22.28	7.81	11.77	26.43	31.52
Etch and Milton	50.85	31.39	39.49	28.16	38.15	30.67
Nothing	33.39	31.89	22.07	27.46	28.72	33.14
Al						
Autoclave	0.70	1.44	1.45	2.96	1.74	2.46
Burnt	1.39	2.35	0.69	1.19	1.27	1.80
Etch and Milton	6.20	4.28	3.75	3.01	2.69	2.20
Nothing	3.54	3.47	1.91	2.54	3.70	4.41
V						
Autoclave	0.27	0.73	0.55	1.32	0.42	0.82
Burnt	0.32	1.06	0.20	0.49	1.09	2.30
Etch and Milton	1.01	1.85	1.41	1.68	2.20	2.87
Nothing	1.04	1.70	1.00	1.50	0.42	1.12
Ca						
Autoclave	1.11	2.07	-	-	2.27	5.89
Burnt	0.16	0.67	1.44	5.83	1.97	4.24
Etch and Milton	0.02	0.08	0.04	0.16	0.18	0.62
Nothing	6.97	18.97	2.93	6.63	3.02	6.94
K						
Autoclave	0.04	0.19	0.13	0.60	0.69	3.08
Burnt	0.02	0.05	0.01	0.04	0.01	0.03
Etch and Milton	-	-	-	-	0.12	0.50
Nothing	0.02	0.05	0.01	0.04	0.12	0.42
Na						
Autoclave	0.14	0.19	0.05	0.12	0.11	0.17
Burnt	0.43	0.67	0.41	0.58	0.32	0.41
Etch and Milton	-	-	0.10	0.42	0.10	0.29
Nothing	0.23	0.42	0.19	0.42	0.18	0.27

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Table 4.3.2 Atomic percentage of elements in areas with visible tissue remnants…Continued

	Areas with Visible Tissue Remnants					
	Body		Neck		Tip	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
P						
Autoclave	0.74	1.42	1.42	4.31	1.55	2.95
Burnt	0.16	0.52	0.16	0.25	1.11	2.69
Etch and Milton	0.46	1.28	0.93	2.26	0.46	1.24
Nothing	2.47	4.72	4.72	6.71	1.71	3.17
S						
Autoclave	0.31	0.59	0.29	0.32	0.16	0.22
Burnt	0.14	0.17	0.28	0.27	0.07	0.11
Etch and Milton	-	-	0.01	0.03	0.05	0.15
Nothing	0.05	0.11	0.44	0.78	0.03	0.07
Si						
Autoclave	2.75	8.33	0.89	3.81	1.13	5.07
Burnt	0.42	1.46	1.11	4.49	0.12	0.33
Etch and Milton	0.09	0.18	3.71	14.77	0.12	0.49
Nothing	0.09	0.24	1.58	6.30	0.02	0.05
Br						
Autoclave	0.01	0.05	0.04	0.20	0.05	0.16
Burnt	-	-	0.11	0.48	0.24	0.96
Etch and Milton	0.01	0.03	-	-	0.57	2.27
Nothing	0.01	0.03	-	-	-	-
Cl						
Autoclave	0.03	0.07	0.00	0.02	0.01	0.06
Burnt	0.04	0.11	0.99	4.03	0.01	0.05
Etch and Milton	2.44	6.32	0.19	0.51	0.32	0.97
Nothing	0.05	0.14	0.48	1.03	0.03	0.08
Fe						
Autoclave	0.03	0.09	0.08	0.30	0.02	0.08
Burnt	0.03	0.08	-	-	-	-
Etch and Milton	-	-	-	-	-	-
Nothing	-	-	0.13	0.51	-	-
N						
Autoclave	4.62	8.04	1.03	3.18	1.41	4.35
Burnt	4.25	7.22	2.97	6.14	3.83	6.27
Etch and Milton	-	-	-	-	0.26	1.04
Nothing	1.90	5.46	-	-	-	-
Mg						
Autoclave	-	-	0.07	0.32	0.02	0.08
Burnt	-	-	-	-	0.01	0.05
Etch and Milton	0.02	0.08	0.42	1.80	0.42	1.69
Nothing	0.08	0.18	-	-	0.03	0.12

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Table 4.3.3 Atomic percentage of elements in areas with no visible tissue remnants

	Areas with No Visible Tissue Remnants					
	Body		Neck		Tip	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
C						
Autoclave	19.35	11.81	17.76	9.79	19.07	12.76
Burnt	17.52	12.26	25.28	21.83	17.36	15.29
Etch and Milton	5.81	5.91	10.14	13.46	5.99	7.50
Nothing	12.36	5.23	13.06	6.20	10.75	9.00
O						
Autoclave	15.46	12.88	13.04	14.01	10.99	12.82
Burnt	16.82	16.66	10.64	15.78	12.80	15.79
Etch and Milton	10.68	17.31	11.47	16.30	13.23	18.78
Nothing	4.85	11.60	4.29	10.15	6.01	12.01
Ti						
Autoclave	55.56	14.73	59.44	15.05	60.64	20.23
Burnt	56.58	20.97	56.39	23.88	60.67	22.82
Etch and Milton	72.76	17.01	67.83	21.50	69.58	20.05
Nothing	71.52	10.32	71.37	10.29	73.42	14.34
Al						
Autoclave	6.89	2.13	7.18	2.04	6.75	2.60
Burnt	6.67	2.32	5.79	3.17	6.87	3.22
Etch and Milton	9.19	2.22	8.04	2.70	8.85	2.55
Nothing	7.93	3.30	7.73	3.07	8.37	1.49
V						
Autoclave	2.09	1.55	2.26	1.80	1.46	1.77
Burnt	2.03	1.58	1.86	1.57	1.41	1.49
Etch and Milton	1.51	1.68	2.51	1.76	2.31	1.76
Nothing	2.78	1.56	3.21	1.29	1.42	1.85
Ca						
Autoclave	0.18	0.29	-	-	0.03	0.09
Burnt	0.07	0.23	-	-	0.05	0.15
Etch and Milton	-	-	-	-	-	-
Nothing	-	-	-	-	-	-
Na						
Autoclave	-	-	-	-	-	-
Burnt	0.07	0.24	-	-	0.12	0.29
Etch and Milton	-	-	-	-	-	-
Nothing	-	-	-	-	-	-
P						
Autoclave	0.19	0.25	0.10	0.31	0.04	0.11
Burnt	0.05	0.12	0.05	0.12	0.07	0.17
Etch and Milton	-	-	-	-	0.04	0.15
Nothing	-	-	-	-	0.04	0.13

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Table 4.3.4 Atomic percentage of elements in areas with no visible tissue remnants…Continued

	Areas with No Visible Tissue Remnants					
	Body		Neck		Tip	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
S						
Autoclave	-	-	-	-	0.02	0.06
Burnt	-	-	-	-	-	-
Etch and Milton	-	-	-	-	-	-
Nothing	-	-	-	-	-	-
Nb						
Autoclave	0.28	0.89	0.22	0.71	0.23	0.74
Burnt	0.08	0.25	-	-	0.30	0.99
Etch and Milton	-	-	-	-	-	-
Nothing	-	-	-	-	-	-
Si						
Autoclave	-	-	-	-	-	-
Burnt	0.11	0.36	-	-	0.18	0.43
Etch and Milton	0.05	0.15	-	-	-	-
Nothing	-	-	-	-	-	-
Br	-	-	-	-	-	-
Autoclave	-	-	-	-	-	-
Burnt	-	-	-	-	-	-
Etch and Milton	0.57	1.79	0.34	1.06	-	-
Nothing						
N						
Autoclave	-	-	-	-	0.77	2.44
Burnt	-	-	-	-	0.17	0.56
Etch and Milton	-	-	-	-	-	-
Nothing	-	-	-	-	-	-

All areas displayed titanium (Ti), aluminium (Al) and vanadium (V), which are the main components of titanium alloy that the mini-implants are made of. All the areas (with and without tissue remnants) also contained carbon (C) and oxygen (O).

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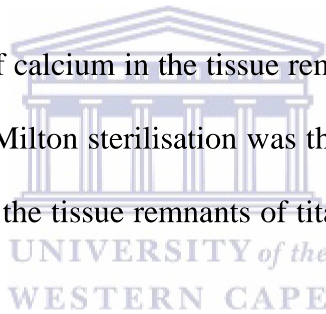
Additional elements were found in the tissue remnants of all the processing groups, namely, Calcium (Ca), Potassium (K), Sodium (Na), Phosphorous (P), Sulphur (S), Silicon (Si), Bromine (Br), Chlorine (Cl), Nitrogen (N), Magnesium (Mg). Iron (Fe) was only found in control, burnt and autoclave groups, however, the etchant and Milton group had no mini-implants with traces of iron.

The atomic percentage of carbon in the tissue remnants of the groups that had been sterilised with heat, namely autoclave and burnt, was almost twice that of the groups that were processes with etch and Milton or had no treatment.

The atomic percentage of calcium in the tissue remnants of the mini-implants that has undergone etch and Milton sterilisation was the lowest of all the groups, with the atomic percentage in the tissue remnants of titanium and aluminium being the highest in these groups.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Small traces of Calcium, Sodium, Phosphorous, Sulphur, Niobium (Nb), Silicon, Bromine, and nitrogen were found in the areas where there were no visible tissue remnants.

4.3.3 Calculation of Percentage Visible Tissue Remnants

4.3.3.1 Repeatability of Measurements

Only one observer performed the measurements on the sample and, therefore, a test was undertaken to ensure that the measurements could be accurately repeated by another observer. An experienced microbiologist measured five randomly chosen images from the main sample. The resulting data were compared with the measurements recorded by the observer on the same five images. The Pearson correlation coefficient of this comparison was 0.998, indicating high agreement between these measurements.

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4.3.3.2 Results of Comparative Test

Percentage visible tissue remnants was determined by the ratio of the area with tissue remnants and the total surface of a two-dimensional representation of each sample.

Table 4.3.5 presents the descriptive statistics of percentage visible tissue remnants per condition. The table shows wide ranges in percentage of tissue remnants for the Gr1, those that had been autoclave, and Gr3, those that had been burnt, and a rather skew distribution for those in Gr2, Etch and Milton, and GrC, where no sterilisation occurred. Gr2 (Etch and Milton) had the lowest percentage visible

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tissue remnants (mean 5.21%); with Gr1 (autoclave) and Gr3 (burnt) having greater percentages of visible tissue remnants (mean 30.08 and 47.04% respectively). The percentage tissue remnants seen in the control group was more comparable to that of the Etch and Milton group (mean 11.07%).

Table 4.3.5 Percentage visible tissue remnants

	Mean	Median	Std Deviation	Min	Max	Skewness
Autoclave	30.08	28.70	16.36	4.06	53.53	0.024
Burnt	47.04	49.94	19.78	3.9	76.97	-0.941
Etch and Milton	5.21	3.74	5.18	0.86	18.23	2.007
None	11.07	8.10	8.46	2.8	29.08	1.128

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Figure 4.3.6 is a boxplot displaying the distribution of percentage tissue remnants for the different methods of sterilisation used in this study. Case number 32, from the Etch and Milton group, was determined to be an extreme outlier. Removal was not required as no statistical analyses were performed on this data.

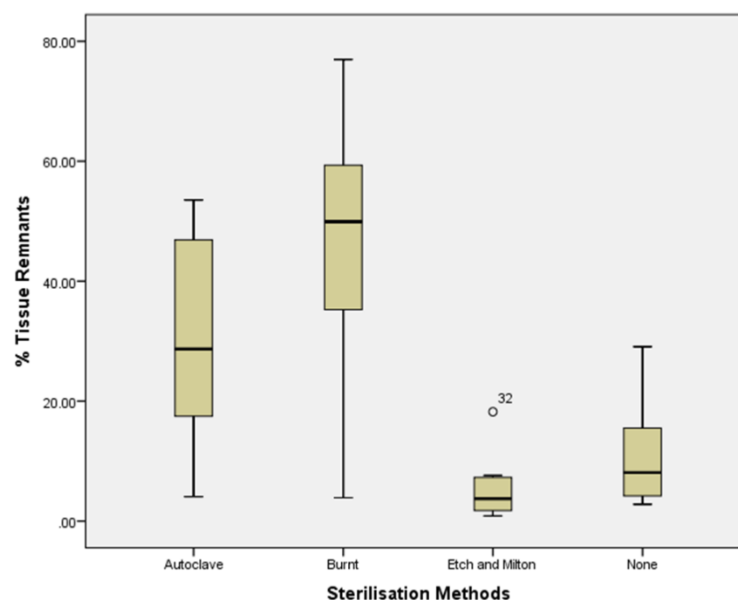

Figure 4.3.6 Boxplot displaying the percentage tissue remnants for each group

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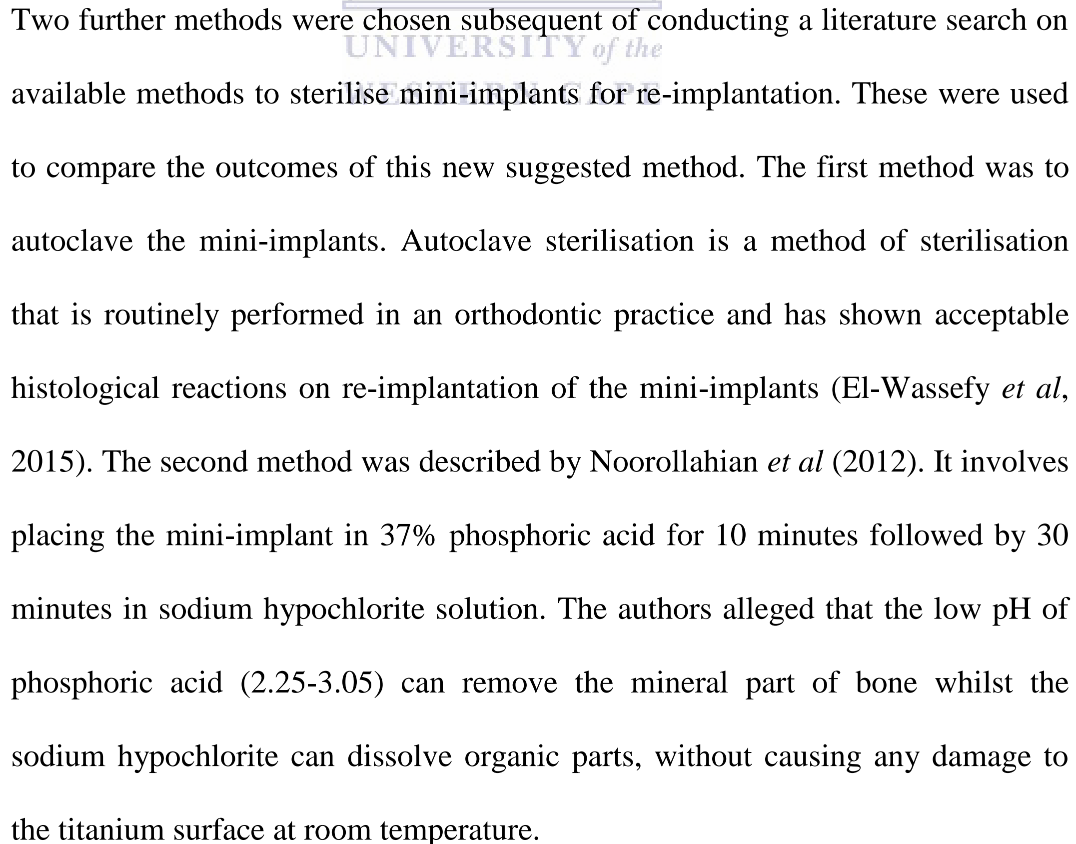
CHAPTER 5: DISCUSSION

Re-implantation of failed mini-implants or relocation is often performed, however, there are no guiding protocols for this treatment design. This study considered a method of sterilising mini-implants that has not been looked at previously in the literature. This method, which has been used in laboratories for sterilising glass rods, involves dipping the mini-implant in 70% ethanol and flaming it (Wright & Harding, 2010). Sterilising in this manner would be ideal in an orthodontic practice as it does not require much time to perform and sterilisation can be achieved during the schedule appointment. This will ensure no additional appointments being required or delay in the overall treatment time.



Two further methods were chosen subsequent of conducting a literature search on available methods to sterilise mini-implants for re-implantation. These were used to compare the outcomes of this new suggested method. The first method was to autoclave the mini-implants. Autoclave sterilisation is a method of sterilisation that is routinely performed in an orthodontic practice and has shown acceptable histological reactions on re-implantation of the mini-implants (El-Wassefy *et al*, 2015). The second method was described by Noorollahian *et al* (2012). It involves placing the mini-implant in 37% phosphoric acid for 10 minutes followed by 30 minutes in sodium hypochlorite solution. The authors alleged that the low pH of phosphoric acid (2.25-3.05) can remove the mineral part of bone whilst the sodium hypochlorite can dissolve organic parts, without causing any damage to the titanium surface at room temperature.

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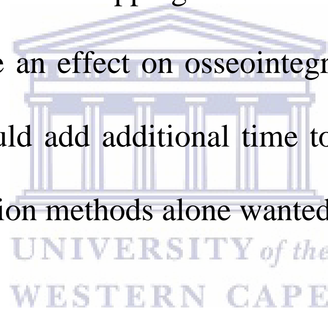
In their study they proved that their method of sterilising prepared mini-implants to a level similar to unused mini-implants (by using calcium ion as an index of tissue remnants). In addition to this, their suggested method of processing mini-implants had no significant difference between the insertion, removal and fracture torques in comparison to unused mini-implants.

The use of cleaning methods prior to sterilising was not included in this study for three reasons: Firstly, due to the possible damage that the cleaning method could have on the mini-implant surface. Sandblasting used to clean mini-implants results in abrasive mechanical stripping of mini-implant surfaces (Pop Silvia *et al*, 2016), which could have an effect on osseointegration. Secondly, addition of an extra step, cleaning, would add additional time to the whole procedure. Thirdly, the effect of the sterilisation methods alone wanted to be tested.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The three methods tested in this study were found to sterilise the mini-implants, with no bacterial growth shown after immersing in a culturing medium. One of the complications involved in re-implantation of a mini-implant includes bacteraemia. As the mini-implants in all groups that underwent sterilisation were proven to be sterile after processing, there should not be an increased risk of bacteraemia to that of an unused mini-implant. The study by Uysal *et al* (2010) showed that there is a slight risk of bacteraemia with the placement of new mini-implants. There is no known reason why this should be higher in the case of re-implantation of sterilised used mini-implants; however, a further study will need to be performed to confirm this. The bacterium found in a blood sample post

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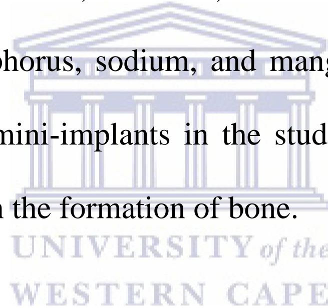
insertion of mini-implant in their study was one that has been shown to be associated with bacterial endocarditis, namely *Streptococcus sanguinis*. They recommended that prophylactic precautions would be necessary in patients that are at risk of such complications. Regardless of whether a new or used mini-implant is used, these precautions should be followed.

Titanium, aluminium, vanadium, carbon and oxygen were among the elements found on all surfaces of the groups. Additional elements were found in the tissue remnants of all the processing groups, namely, calcium, potassium, sodium, phosphorous, sulphur, silicon, bromine, chlorine, nitrogen, magnesium. The elements calcium, phosphorus, sodium, and manganese, and nitrogen were also found on the retrieved mini-implants in the study by Eliades *et al* (2009), and were said to be present in the formation of bone.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The appearance of the tissue remnants on the mini-implant surfaces varied amongst the groups with areas of elevated tissue remnants and smoother discoloured areas. The darkening/discolouration seen on the retrieved mini-implant surfaces was also seen in a study (Vezeau *et al*, 1996) looking into the effect of autoclave sterilisation on unused commercially pure titanium discs. The authors of this study stated that this discolouration could be due to a surface oxide change or a contaminant, such as iron, that may have been deposited. They assumed that iron, sodium, phosphorus, and silicon which were found on the surfaces were due to purity of the water used to prepare the steam. This explanation does not seem to apply to this study as the mini-implants were placed

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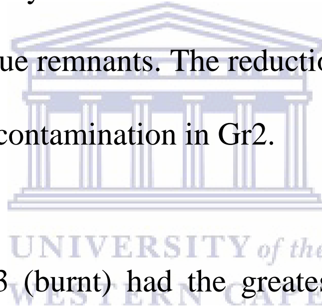
in sealable packets prior to autoclaving and impurities in the water would not be able to cause deposition on the mini-implants.

It was evident on the scanning electron microscopy images and through the element analysis that the three methods of sterilisation differed in their effect. The mini-implants in Gr2, that were sterilised by etch and Milton, showed no traces of iron (which were present in the other groups) and the atomic percentage of calcium in the tissue remnants was the lowest of all the groups. Eliades *et al* (2009) said that calcium and iron deposits on mini-implant are caused by contact with biologic fluids, mostly blood. Noorollahian *et al* (2012) also used calcium ion as an indicator of tissue remnants. The reduction of calcium and lack of iron is an indication of reduced contamination in Gr2.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Gr1 (autoclave) and Gr3 (burnt) had the greatest percentages of visible tissue remnants (mean 30.08 and 47.04% respectively) in comparison to Gr2 (etch and Milton) which had the lowest/minimal percentage visible tissue remnants (mean 5.21%). Additionally, these methods of sterilisation, Gr1 and Gr3, had almost double the atomic percentage of carbon compared to Gr2 and GrC. As mentioned previously, the phosphoric acid dissolves inorganic material, followed by the Milton that would remove the organic material. This chemical method of cleaning provides better removal of the tissue remnants. Whereas the other methods of sterilising the mini-implants have no attempt at removing the tissue remnants, and during the sterilising process they are exposed to heat which results in this “carbonised residue” remaining on the surface.

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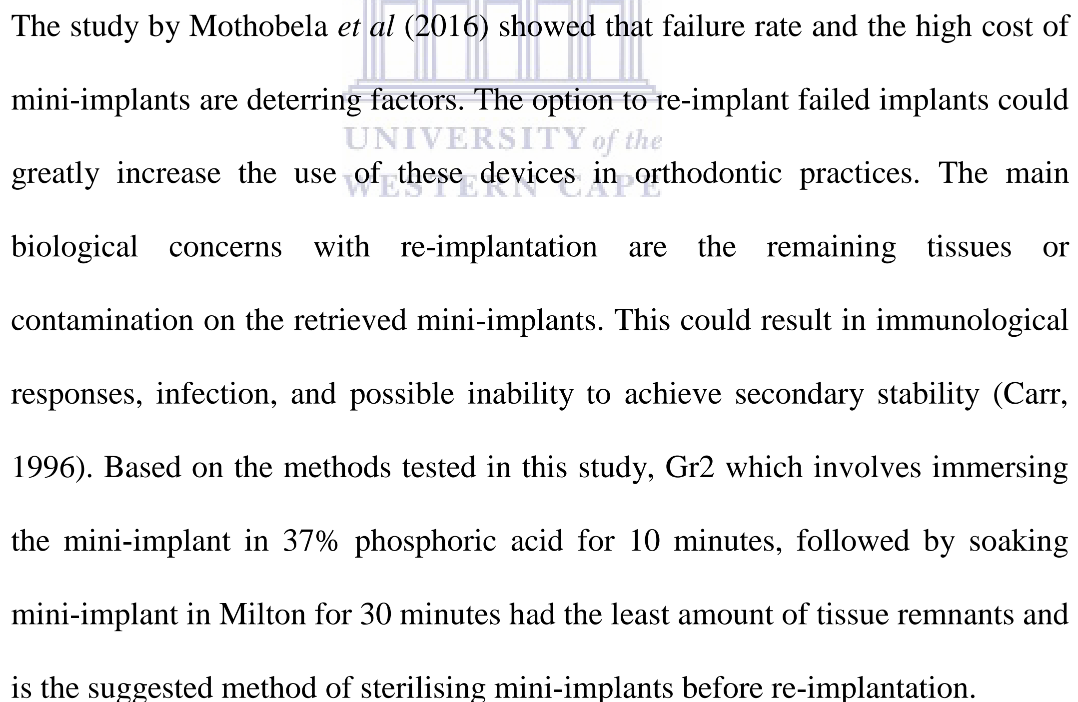
The mini-implants from the control group, GrC, had less visible tissue remnants to that of Gr1 and Gr3. This was an unexpected finding and the best possible explanation for this was that other variables may have resulted in this finding. Due to the limited sample size, variables such as brand, location of placement of mini-implant, reason for removal, length of service in the mouth, and orientation of the mini-implant for imaging could not be evaluated to determine whether they had an effect on the outcome of sterilisation. This may have influenced the results in this study. A future study should either reduce these variables or obtain a larger sample size in order to examine the effect of these variables. This was, however, not possible in this study due to time constraints.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The study by Mothobela *et al* (2016) showed that failure rate and the high cost of mini-implants are deterring factors. The option to re-implant failed implants could greatly increase the use of these devices in orthodontic practices. The main biological concerns with re-implantation are the remaining tissues or contamination on the retrieved mini-implants. This could result in immunological responses, infection, and possible inability to achieve secondary stability (Carr, 1996). Based on the methods tested in this study, Gr2 which involves immersing the mini-implant in 37% phosphoric acid for 10 minutes, followed by soaking mini-implant in Milton for 30 minutes had the least amount of tissue remnants and is the suggested method of sterilising mini-implants before re-implantation.

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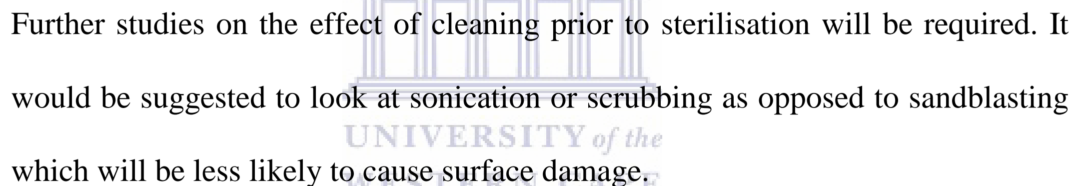
Only 2 (5%) of the retrieved mini-implants in this study were removed due to failure, which is lower than the overall failure rate of 13.5% shown in the meta-analysis performed by Papageorgiou *et al* (2012). A possible explanation for this may be that a clear definition on what failure includes was not provided to the practitioners. Failure includes anything resulting in the inability of the mini-implant to act as a stationary anchor and/or necessitates its removal or replacement (Papageorgiou *et al*, 2012). This could include even slight mobility which would often be overlooked and not defined as failure by the practitioner. The failure rate may, therefore, be understated in this study. No association to site of placement or practitioner was found.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Further studies on the effect of cleaning prior to sterilisation will be required. It would be suggested to look at sonication or scrubbing as opposed to sandblasting which will be less likely to cause surface damage.

Additional studies would be required to examine whether these methods of sterilisation affected the properties of the metal, which could result in increased ion release or changes in the histological response on re-implantation. A cell attachment and morphology study making use of fibroblasts or osteoblasts (to generically determine cell responses at the interface) or an animal study to view the histological response around the mini-implant will be required to determine what the biological result would be to re-implantation of retrieved, sterilised mini-implants.

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CHAPTER 6: CONCLUSIONS

All processing methods examined were able to sterilise the mini-implants tested and no bacterial growth was present after culturing in Brain Heart Infusion Medium.

There were, however, differences in their surface appearances. Gr2, etch and Milton, displayed the least amount remaining surface remnants (mean 5.21%), whereas Gr1 (autoclave) and Gr3 (burnt) showed mean of 30.08 and 47.04% tissue remnants on their surfaces respectively.

Titanium, aluminium, vanadium, carbon and oxygen were found on all surfaces of the groups. Additional elements were found in the tissue remnants of all the processing groups, namely, calcium, potassium, sodium, phosphorous, sulphur, silicon, bromine, chlorine, nitrogen, magnesium. Sterilising methods autoclave and burning (Gr1 and Gr3), which made use of heat, had almost double the atomic percentage of carbon. Gr2, etch and Milton, had the lowest atomic percentage of calcium and none of the mini-implants in this group showed traces of iron, which was present in the other groups.

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Based on the methods tested in this study, Gr2 which involves immersing the mini-implant in 37% phosphoric acid for 10 minutes, followed by soaking mini-implant in Milton for 30 minutes is the suggested method of sterilising mini-implants before re-implantation.

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Further studies looking into additional sterilisation methods, possibly with the use of sonication or scrubbing, are required to draw up protocols on re-implantation of mini-implants. Additional histological and ion release tests will also be required to confirm which method of sterilisation will result in no additional complications in the patient to that of insertion of a new, unused mini-implant.

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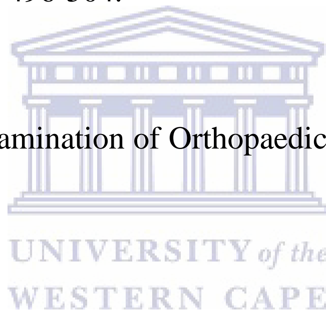

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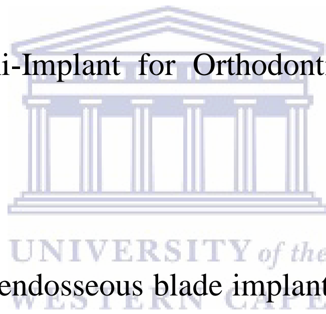

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ADDENDUM A

<u>RETRIEVED MINI-SCREW</u>

Practitioner name:___________________

Date & Time of removal:______________

Date placed:_______________________

Patient:

DOB:________________________

Gender:______________________

Race:________________________

Implant:

Brand:_______________________

Size:________________________

Location placed:_______________

Reason for removal:__________

PLEASE PLACE IN FRIDGE IMMEDIATELY

AND CONTACT TARYN 0825082255

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ADDENDUM B

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BRAIN HEART INFUSION

Code: CM225

A highly nutritious infusion medium recommended for the cultivation of streptococci, pneumococci, meningococci and other fastidious organisms. Suitable for blood culture work.

Formula	gm/litre
Calf brain infusion solids	12.5
Beef heart infusion solids	5.0
Proteose peptone	10.0
Glucose	2.0
Sodium chloride	5.0
Disodium phosphate	2.5
pH 7.4 ± 0.2	

Directions
Dissolve 37g in 1 litre of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes.

Description
A versatile liquid infusion medium which is suitable for the cultivation of streptococci, pneumococci, meningococci, and other fastidious organisms. This medium is recommended for blood culture work and, with the additions described below, for the isolation and cultivation of pathogenic fungi.

Oxoid Brain Heart infusion is essentially a buffered infusion broth giving similar results to the brain dextrose broths originally employed for the cultivation of streptococci[1], and for the cultivation of dental pathogens[2].

The addition of 0.1% of agar will serve to reduce convection currents and so create conditions of varying oxygen tension which favour the growth and primary isolation of aerobes and anaerobes[3], while even easily cultivated organisms show improved growth[4].

Brain Heart Infusion was used in a test for the pathogenicity of streptococci and the same medium was enriched with ascitic fluid for the cultivation of gonococci.

Oxoid Brain Heart Infusion is especially useful as a growth and suspension medium for staphylococci which are to be tested for coagulase production; Newman[8] employed a similar, medium for this purpose in an investigation of food poisoning caused by dairy products.

A satisfactory medium for blood culture can be prepared by adding 1g of agar per litre of Brain Hear Infusion. Ensure that the agar is uniformly distributed in the sterile broth before dispensing into bottles. More conveniently, add 1 Agar Tablet CM49 to each 100ml of Brain Heart Infusion and sterilize by autoclaving for 15 minutes at 121°C. Cool to 60–70°C and mix gently to ensure uniform distribution of the agar.

Tubes of Brain Heart Infusion which are not used the same day as sterilized should be placed in a boiling water bath for several minutes to remove absorbed oxygen, and cooled rapidly without shaking, just before use.

Further supplements to improve the recovery of organisms from blood can be added before sterilization or aseptically post-sterilization. Co-enzyme 1 (NAD), penicillinase and p-amino benzoic acid are examples.

Brain Heart Infusion supplemented with yeast extract, haemin and menadione was consistently better in producing heavy growth of five species of *Bacteroides* than three standard anaerobic broths. Furthermore, microscopy of overnight cultures showed normal morphology in Brain Heart Infusion but abnormal morphology in the three anaerobic broths[9].

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store tubed or bottled medium in the dark and below 20°C.

Quality Control
Positive controls:
Streptococcus pneumoniae ATCC® 6303
Candida albicans ATCC® 10231

Negative control:
Uninoculated medium.

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ADDENDUM C

Office of the Deputy Dean for Research
Faculty of Dentistry & WHO Collaborating Centre for Oral Health
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Private Bag X1, Tygerberg 7505
Cape Town
SOUTH AFRICA

Date: 30th November 2015

For Attention: Dr di Pasquale (St. No. 2434226)
Department of Orthodontics
Faculty of Dentistry
Tygerberg Campus

Dear Dr di Pasquale

STUDY PROJECT: Evaluation of sterilising methods for re-implantation of orthodontic mini-implants

PROJECT REGISTRATION NUMBER: 15/7/29

ETHICS: Approved

At a meeting of the Senate Research Committee held on Friday 27th November 2015 the abovementioned project was approved. This project is therefore now registered and you can proceed with the study. Please quote the above-mentioned project title and registration number in all further correspondence. Please carefully read the Standards and Guidance for Researchers below before carrying out your study.

Patients participating in a research project at the Tygerberg and Mitchells Plain Oral Health Centres will not be treated free of charge as the Provincial Administration of the Western Cape does not support research financially.

Due to the heavy workload auxiliary staff of the Oral Health Centres cannot offer assistance with research projects.

Yours sincerely

Professor Sudeshni Naidoo

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