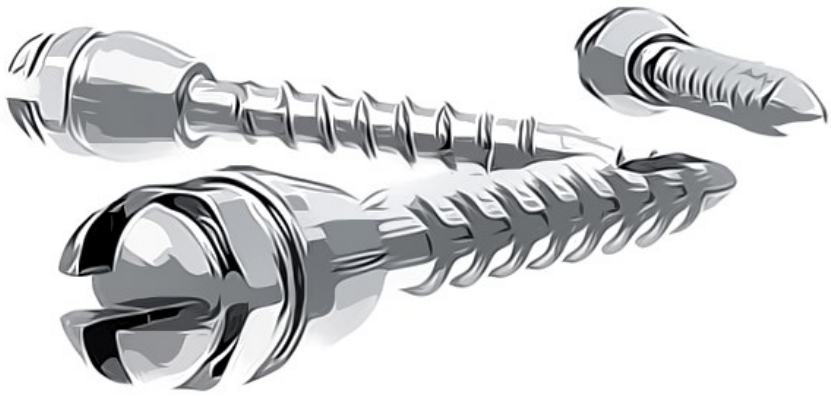
The effect of surface characteristics and antimicrobial agents on the growth of biofilms on **Orthodontic mini-screw implants**

Thesis for partial fulfilment of the requirements for a Doctor of Clinical Dentistry in Orthodontics.



Shaneel Shastri

JAMES COOK UNIVERSITY
AUSTRALIA

The effect of surface characteristics and antimicrobial agents on the growth of biofilms on **Orthodontic mini-screw implants**

Thesis for partial fulfilment of the requirements for a Doctor of Clinical Dentistry in Orthodontics.

Shaneel Shastri

College of Medicine and **Dentistry** | Department of **Orthodontics**

Submitted: March 2015

Table of contents

List of abbreviations

Al	Aluminium
ANOVA	Analysis of variance of mean
AFM	Atomic force microscopy
ASOFRE	Australian Society of Orthodontists Foundation for Research and Education
CHX	Chlorhexidine
F	Fluoride
FCS	Flow Cytometry Standard
OMSI	Orthodontic mini screw implant
PMSF	Phenylmethanesulfonylfluoride
PI	Propidium iodide
SEM	Scanning Electron Microscopy
TAD	Temporary anchorage device
TO	Thiazole orange
Ti–6Al–4V	Titanium alloy (Titanium, Aluminium, and Vanadium)
V	Vanadium

List of figures

List of tables

Declaration

I, Dr. Shaneel Shastri, do solemnly and sincerely declare that this thesis has not been accepted for the award of any other degree or diploma in any other University.

To the best of my belief, it contains no material published, except where due reference is made in the text. I give consent for this copy of my thesis, when deposited in the university Library to be made available for loan and photocopy. Every reasonable effort has been made to gain permission and acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.

Dr. Shaneel Shastri BDSc.(Melb) FRACDS Date:

Tuesday, June 09, 2015

Declaration of ethics

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the National Statement on Ethics Conduct in Research Involving Humans (1999), the Joint NHMRC/AVCC Statement and Guidelines on Research Practice (1997), the James Cook University Policy on Experimentation Ethics, the Standard Practices and Guidelines (2001) and the James Cook University Statement and Guidelines on Research Practice (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee. (See Appendix A for copy of letter and approval number)

Acknowledgement

Professor Adam Qingsong Ye and Professor Andrew Sandham, for all their encouragement and guidance throughout my graduate study.

Dr. Kate Miller and Dr. Yan He for their technical support and advice.

James Cook University for the kind loan of materials and resources utilized for this Thesis.

To the Australian Society of Orthodontists Foundation for Research and Education (ASOFRE) for their generous support in funding part of this research.

Staff and graduate students of Orthodontics, James Cook University for their support in completing this thesis. Last but definitely not least, my family and friends for their unfailing support in all I do.

Statement on the contribution of others

Contribution	***Name***
Assistance with proposal writing and project development	Professor Andrew Sandham Professor Adam Ye
Laboratory support and assistance	Dr. Kate Miller Dr. Yan He
Assistance with Data collection	Dr. Shane Askew Dr. Jen Wilkinson Dr. Paul Giacomin Fred Carlstrom
Assistance with statistical support and interpretation	Dr. Susan Jacups
Professional editorial assistance	Dr. Don Gilchrist
Financial support	ASOFRE

Abstract

Background:

Orthodontic mini-screw implants (OMSIs) have become largely popular in contemporary orthodontics to attain anchorage. However, a high rate of failure of these devices has been one of the disadvantages. One of the potential areas for failure that have been identified may be the aggregation of biofilm of these devices leading to peri-implant inflammation[9]. To date, the investigation into the various surface characteristics of various OMSI systems has been limited. Surface roughness has been identified as a factor involved in biofilm aggregation[10].

Aim:

This study aims to compare the effect of surface characteristics and antimicrobial agents on the growth of biofilms on orthodontic mini-screw implants. The null hypothesis states that there will be no difference in the surface roughness or biofilm growth between implant groups. Furthermore, there will be no difference in the efficacy of antimicrobial agents.

Method and materials:

Four OMSIs with various surface finishes available in the Australian market were selected for the present study. They were: Vector – Anodised titanium alloy, TOMAS – machined titanium alloy, Leone – Stainless steel, and Aarhus Anodised titanium alloy. Five implants from each group were selected and tested under atomic force microscopy with a INTEGRA Modular AFM (NT-MDT, Moscow, Russia) and scanning electron microscopy Jeol JSM5410LV (Peabody, Massachusetts, USA) around the head and neck region. Each implant was measured at three random sites. Qualitative and quantitative data were collected for statistical analyses.

In a separate arm to the experimental process, biofilm was cultured around each implant group with flow cytometry utilized to determine which implant groups were more conducive to biofilm growth. Antimicrobial agents including Chlorhexidine, and Fluoride were also investigated to determine the effect these would have on biofilm grown on the selected OMSI groups.

Results and Discussion:

One way ANOVA revealed that between the implant groups, Vector OMSIs were shown to have the roughest surface (Mean surface roughness of 239.4 ± 71 nm ($p<0.00$). The roughness of the other groups showed no statistical significance. These findings were reflected in the SEM and bacterial studies which showed the Vector group to have a higher surface roughness as well as leading to increased biofilm growth. Interestingly, the Aarhus group (Mean surface roughness of 127.8 ± 30 nm ($p<0.00$), whilst not as rough as the Vector group also displayed increased biofilm growth. When comparing antimicrobial efficacy, Chlorhexidine was shown to be greater then twice as effective as fluoride when used as an antimicrobial agent in controlling biofilm around OMSI.

Conclusions:

This study shows that when investigating OMSI, increased surface roughness may result in increased biofilm growth. Anodized titanium alloy surfaces were found to be both the roughest, and most conducive to biofilm grown. This may be a factor in the operator choosing an appropriate OMSI system for use in their patients to limit the amount of biofilm growth. When comparing antimicrobial agents, chlorhexidine remains a better choice to effectively clean OMSI *in situ.*

Chapter 1

Literature review

1.1 Introduction

Orthodontics like many specialist facets of dentistry has benefited from the development and subsequent implementation of various technologies in clinical practice. Primitive and surprisingly well designed orthodontic appliances have been found with Greek, and Etruscan artefacts [11] [12]. Archaeologists have even discovered Egyptian mummies with rudimentary precious metal bands wrapped around individual teeth, secured with what appeared to be cat gut wrapped around individual teeth[13].

Traversing all of these millennia, while the fundamental principals of orthodontics remains, it seems our armamentarium now consists of self-ligating brackets, sequential clear aligners, cone beam computed tomography, distraction osteogenesis, bioengineering, robotically bent arch wires, and implants used as temporary anchorage devices (TADs) [14].

In particular, the usage of TADs and their implementation in orthodontics stems from the popularisation of implantology in restorative dental practice [15]. Since the pioneering work of Brånemark [16] [17] [18] in the last century, the phenomenon of osseointegration has been studied and followed up in great detail[19].

His definition of a direct contact between living bone and an implant, on the light microscope level describes the objective of osseointegration, but the essence of its clinical success is the reliability of long-term implant fixation, even in the presence of functional loading. This has been corroborated by various studies, reporting high success rates [20] [21].

Such pioneering work has translated to clinical outcomes where it is possible to replace teeth with titanium analogues supporting homogenous or heterogeneous crowns. In addition, this technology is also being utilised not only to replace single teeth [22] [20] but multiple teeth [23] [24], and in some cases entire arches [25] [26].

Despite the exciting applications of dental implantology in restorative dentistry, this field is also able to offer much to the discipline of orthodontics.

During active orthodontic treatment, concept of anchorage aims to limit the extent of detrimental, unwanted tooth movement. It has been defined as "resistance to undesired tooth movement" [27]. This notion of differential tooth control is one that has plagued orthodontics from its very inception, and proves to be difficult to regulate.

Edward Angle [28] referred to the famous postulations of Newton to state that ; *"According to the well-known law of physics, action and reaction are equal and opposite, hence it must follow that the resistance of anchorage must be greater than that offered by the tooth to be moved, otherwise there will be displacement of the anchorage and failure in the movement of the teeth to the extent, or possibility, in the direction desired. The sources at our disposal for securing anchorage or resistance are, first, the teeth, themselves, and second, sources external to the teeth."*

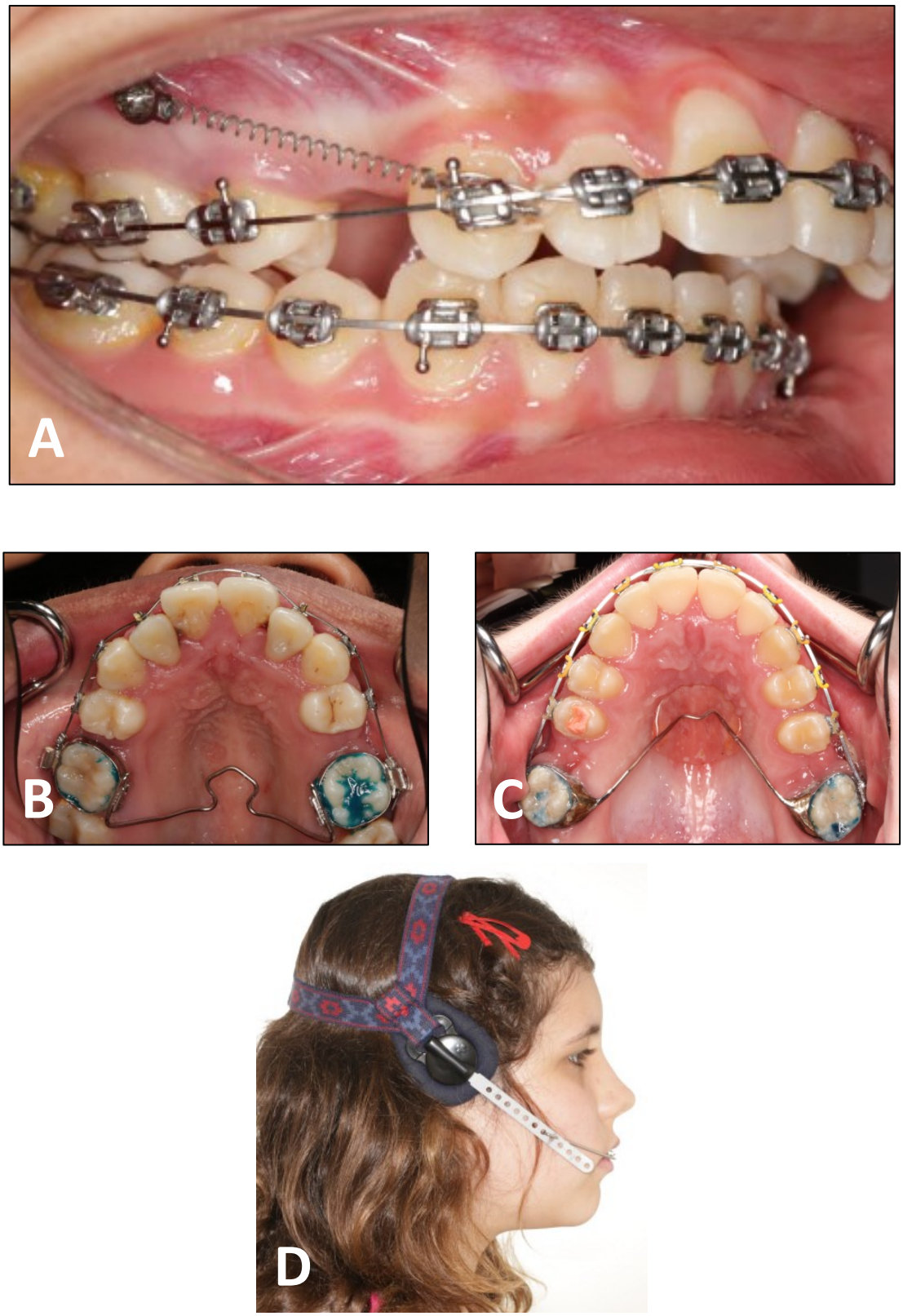


Figure 1.1 : Orthodontic devices to assist in anchorage control.

A – OMSI, B – Transpalatal arch, C – Nance appliance, D - Headgear.
Source : Dr. Shaneel Shastri

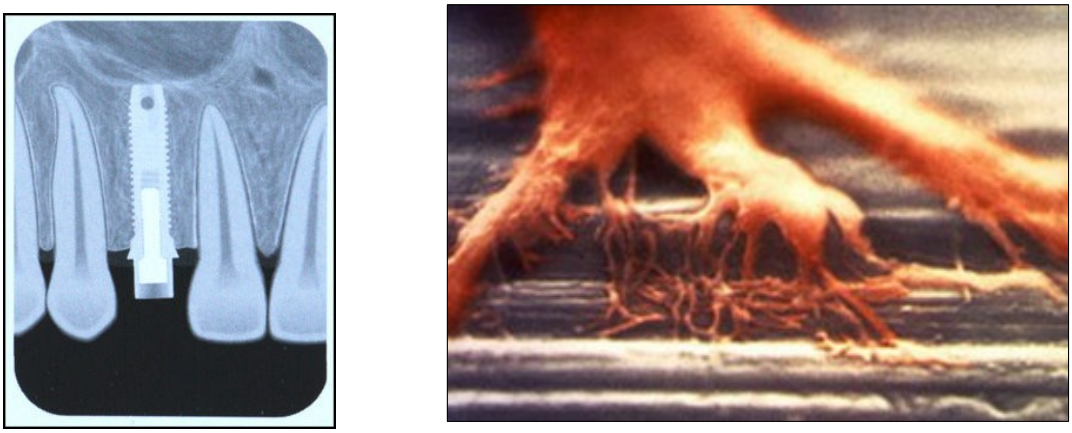


Figure 1.2 : Implants in dentistry:

A – Radiograph demonstrating an osseointegrated Brånemark fixture in vivo.
B – High power view of cellular processes growing onto a titanium surface.
Source : www.branemark.com [3]

1.2 The concept of absolute anchorage

To this day, anchorage remains a challenging concept in orthodontics and since Angle's work, a more intricate classification has been proposed by others including;

- Simple, reciprocal, inter-maxillary or extra oral (Ottofy [29])
- Single, compound or reinforced (Moyers [30])
- Maximum, moderate or minimum (Gianelly and Goldman [31])
- A, B or C type anchorage (Marcotte [32] and Burstone [33])

Traditionally, orthodontics has been limited by our control of intraoral anchorage as reciprocal force expression inevitably leads to unwanted tooth movements. This necessitates prolonged treatment as time is spent undoing or controlling the sequelae. Consequently, the concept of *absolute anchorage* developed which describes zero anchorage loss on the reactive unit. This would imply implements that were not subject to the laws of physiological tooth movements.

Consequently, the skeletal system is described as the most plausible solution to gaining such an effect, and thus the term *skeletal anchorage* used sometimes synonymously, implying the same connotations as absolute anchorage.

Anatomically there are few locations intra-orally which can facilitate such an outcome. Perhaps the ankylosed tooth is a conventional and easily adaptable example of skeletal anchorage [34] [35].

Extra oral devices such as headgear have been used successfully in the past to provide skeletal anchorage by transferring reactive forces to the head and neck [12,36] [37] [38]. However these systems are often difficult to utilise and often rely almost completely on patient compliance.

These appliances consist of respective intraoral and extra oral components. Generally speaking, the intraoral components attached to the teeth and jaws through a combination of bands on the upper molars, and some type of baseplate if used in conjunction with a functional appliance. The extra oral components include a face bow and skeletal stabilization [12]. Although the intraoral components are cemented to the teeth, the chance that they may shift is possible, though affecting the force vectors. This can occur through regular usage, diet, and/or physical trauma [39] [40].

In addition, to be of any benefit, headgear is recommended to be worn for a minimum of 10 to 12 hours per day [12]. These guidelines are perhaps formulated more to fit in with social appearances of patients rather than actual science.

Ideally, to have significant clinical effect headgear should be worn for 23 hours per day [41]. Thus, since the inception of headgear, patient compliance has been the major drawback of its effectiveness [42] [43]. Furthermore, the extra oral components of these devices are unsightly and potentially uncomfortable, especially while sleeping. It has been shown that such psychological, social and physical factors hinder patient compliance [44] [45].

Studies using headgear timers have shown that patients wear their headgear for less than 50% of the time that is actually prescribed by the clinician [46] [47]. Growth is also a prerequisite for headgear treatment with younger patients who have not ceased growing the most suitable. It is even more challenging to gain compliance with this age group of people as they are often subject to complex social and psychological interactions during these formative years.

In addition, the force applied through these devices is often much greater than desired. The practitioner is required to carefully consider the force in relation to desired treatment outcomes. Oversight may result in inadequate or extreme forces leading to undesired outcomes or potentially even injury.

The work of Samuels [48] showed that significant injury may occur through the use of headgear, some as serious as loss of eyesight.

Whilst recently, locking face bows have become available, which are much safer; [49] when headgear injury does happen the results can be catastrophic.

The pursuit for more predictable, effective, and acceptable methods of treatment has led to the development of TADs which fulfil these requirements. TADs attempt to provide absolute anchorage, and allow opportunities to a new field of biomechanics with which better patient outcomes may be delivered.

This notion of skeletal anchorage is a paradime shift as it forces us to revise traditional biomechanics in orthodontics [50]. Tasks that were not possible or immensely difficult previously, have become plausible if not possible. For example, some of the possibilities of skeletal anchorage allow us to [51]:

- Retract and realign anterior teeth without posterior support
- Close edentulous spaces in molar extraction sites
- Correct centreline discrepancy when missing posterior teeth
- Re-establish transverse and antero-posterior position of isolated molar abutments.
- Stabilise teeth with reduced bone support
- Utilise orthodontic mechanics as an alternative to an orthognathic surgical procedure

1.3 The development of TADS in orthodontics

The notion of skeletal anchorage is not a new concept. Recognising the limitations of traditional methods of anchorage in 1945, Gainsforth and Higley [52] placed vitallim screws in the ascending ramus of dogs to orthodontically move their canines. They found that all screws were lost with a range of survival from 16 – 30 days. Whilst they concluded that the development of such a method of anchorage was hopeful, they were sceptical as to the stability of the screws, and the risk of infection.

Furthermore, the first clinical implementation of a TAD was reported almost 40 years later in the literature by Creekmore and Eklund [53] in 1983. Once again, Vitallium bone screws were chosen to be inserted into the anterior nasal spine to treat a patient with a deep overbite.

With growing interest in the area, in 1969, Linkow placed blade implants to rubber bands rubber bands used to retract teeth. Unfortunately, he never presented any long term results [54].

In 1984, Roberts corroborated the use of implants in orthodontic anchorage [55]. Following a delayed loading protocol, titanium screws were loaded for 4 to 8 weeks by stretching a spring between the screws. A high success rate was presented with all but 1 of 20 implants remaining rigid.

With growing momentum in the area, this paved the way for a myriad of subsequent studies which focused on the use and development of other means to obtain skeletal anchorage for orthodontic tooth movement. Among the investigated methods were:

- Conventional dental implants [56 55 57 58 59]
- "Onplants" [7 60 61 62 63 64]
- Miniplates [65 66 67 68 69]
- Zygoma wires [70]
- Palatal implants [71]
- Orthodontic mini-screw implants [72 73 74]

It should be noted that the ideal properties of an implant used to enhance orthodontic anchorage have been described by Ismail, and Johal [75] as being ;

- Biocompatible
- Inexpensive
- Easily inserted and removed under local anaesthetic
- Small enough to locate in multiple sites of the mouth
- Osseointegrate rapidly
- Stable to orthodontic loading in all planes of space.

Furthermore Cope [35] classified the current available methods of skeletal anchorage as either bio compatible or biologic in nature. The biologic group included ankylosed and dilacerated teeth, whereas the biocompatible group included TADs. Further sub-classification was made to describe the manner in which they are attached to bone into biochemical (osseointegrated) or mechanical. Perhaps Labanauskaite [76] provides the most thorough classification of implants used for orthodontic anchorage;

According to the shape and size	**Conical (Cylindrical) i.e. Screws**	**Flat implants i.e. Mini Plates**
According to the implant bone contact	**Osseointegrated**	**Non-osseointegrated**
According to the application	- Used only for orthodontic purposes (Orthodontic implants) - Used for prosthodontic and orthodontic purposes (Prosthodontic implants)	

Table 1.1 : Classification of temporary anchorage devices.
Source **:** Adapted from Labanauskaite 2005 [76].

Conventional dental implants have also been used for orthodontic purposes[57] [58] [59]. In some instances where tooth replacement may be required as the end goal, this process is valuable as it allows the implants to play a dual role. Firstly, as a source of skeletal anchorage to help ascertain effective tooth movement [77]. Secondly as a foundation for tooth replacement at the conclusion of treatment [78].

Interestingly, Kokich [79], Smalley [80] and Blanco [81] have described protocols to determine accurate dental implant placement, ensuring their usability in the final desired location for restorative procedures. However their implementation remains difficult and prediction let alone control of space requirements are more dynamic than precise. Especially when tolerances with conventional dental implant planning are very small (0.5 – 1.5mm), this is usually a perilous task with zero margin for error.

In addition, one of the obvious disadvantages of two stage implants for orthodontic anchorage is the need for a protracted healing period (Commonly 3-6 months [82]), which contributes significantly to the treatment time. In addition, due to their generally enlarged dimensions in comparison to the orthodontic counterparts, conventional dental implants often require increased bone volume to facilitate their placement.

As a result of these inherent limitations, this furthered the need to design specific implants for orthodontic purposes.

Block and Hoffman [83] proposed a disc like structure referring to it as an 'onplant'. The onplant is a distinctive structure as it engages bone in a unique manner. It is a hydroxyapatite coated disk which is 10mm and diameter, and 3mm thick. The surgical placement involves adopting a tunnelling approach to the posterior aspect of the hard palate where the disk is secured by a smaller central screw. After a 10 - 12 week healing period, loading consists of connection of the onplant via orthodontic bands to the upper teeth by a transpalatal arch. It has been shown that this mechanism may resist greater than 300g of continuous orthodontic force [7], which is comparable to the force required in orthodontic systems to close extraction spaces. Following treatment, the onplant may be removed with an osteotome although many authors have raised objections to the possible clinical complications, and acceptance of this method by patients. Although the onplant addressed the issue of bone volume with respect to implant placement, there are numerous pitfalls including; a complex surgical procedure, a protracted healing time, limitations in where the device may be placed, and a traumatic retrieval process which may be disconcerting to the patient.

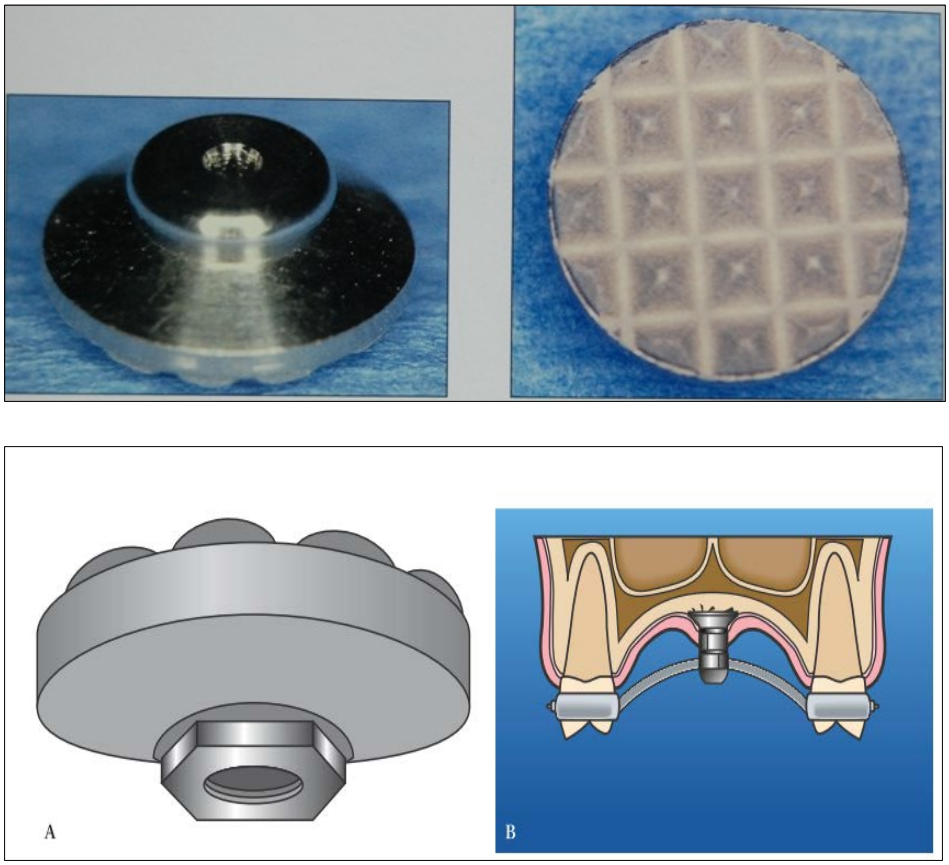


Figure 1.3 : Onplant type TADs.

A - Illustration of an onplant (Nobel Biocare, Göteborg, Sweden) as described by Block and Hoffman[7].

B - Diagram of placement of the onplant connected to a transpalatal arch.

Source : Jaggi 2012 [8].

Another novel method to gain skeletal anchorage was advocated by Melsen [70]. In extreme cases where no other solutions may be found, the region of the zygomatic arch and infra-zygomatic crest may be utilised in a method to loop ligatures around these structures to gain maxillary anchorage.

To utilise this method, it is suggested to drill a horizontal canal 1.0 cm lateral to the alveolar process. The entrance and exit holes should be in the superior portion of the infrazygomatic crest. Melsen used a 0.12 inch stainless steel ligature threaded through this canal and exposed into the oral cavity. The wire was loaded immediately as there was no need to wait for integration. When the ligatures are no longer needed, they may be removed under local anaesthesia as would sutures. This technique is inexpensive, does not require special equipment, and anchorage can be used immediately. However, there are anatomical limitations in the design of mechanics, as well as a potentially significant surgical approach to install the wires. In addition, the proximity to the orbit may be dangerous if infection were to track into these peri planar facial planes. Melsen has also noted that often when treatment was prolonged, the described zygoma wires migrated occlusally through bone! It is only Melsen who has described the use of zygoma wires in the literature.

Titanium miniplates similar to that used in the fixation of bone have also been reported in the as another means of skeletal orthodontic anchorage [9 84 85 86 87]. Umemori [88] installed these miniplates using bone screws on the buccal cortical surfaces of the lower molars. Elastic threads were utilised to successfully intrude the lower molars to help correct open-bite malocclusion. This method proved to be simple, effective, and available almost immediately.

Most recently, the work of De Clerke showed that mini plates may be used for maxillary protraction in Class III skeletal corrective treatment [89 90 91 92 93].
It has been suggested that mini plates provide a high success rate comparable to that of orthodontic mini-screw implants [94 76], if not greater [95]. Therefore, as far as stability is concerned, mini plates have an advantage over mini screw implants. In addition, for certain orthodontic movements requiring large forces, Umemori and Sherwood have suggested using mini plates on the grounds of their extensive stability and the ability to sustain larger forces [87 66].

However, to install them, miniplates need to be secured on multiple areas of cortical bone, and to cover the body of the plate sub-periosteally, a significant surgical procedure is required to install them. This requires soft tissue trauma, and hence operative, and post-operative discomfort [96 97,98].

Interestingly, Cornelis and De Clerke [99] found that patient's perception of mini plates including the postoperative discomfort was not significant comparing it to that of having restorative dental treatment completed. In addition, there are anatomic limitations in sites where miniplates may be considered making them less versatile than other types of TADs.

Triaca [100] was perhaps the first to consider the anterior region of the hard palate as a potential site for orthodontic implant placement. In addition, Wehrbein and Merz [101] further investigated this suggested anatomical location by measuring the depth of bone in the mid-palatal on lateral cephalograms. Subsequently, the Straumann Orthosystem *(Institut Straumann AG, Waldenburg, Switzerland)* implant system was developed. The Orthosystem implant consists of a variable length, screw-type endosseous section, a cylindrical transmucosal neck and an abutment, to which a transpalatal arch attaches.

Although simplified to the method of Block and Hoffman [7], the individual variation in depth and width of the mid-palatal suture, as well as proximity of the nasal floor in children, means that this location may not always be appropriate. In addition, the loading protocol is delayed in order to allow osseointegration, thus anchorage is not immediately available, nor is it adaptable to other sites in the oral cavity.

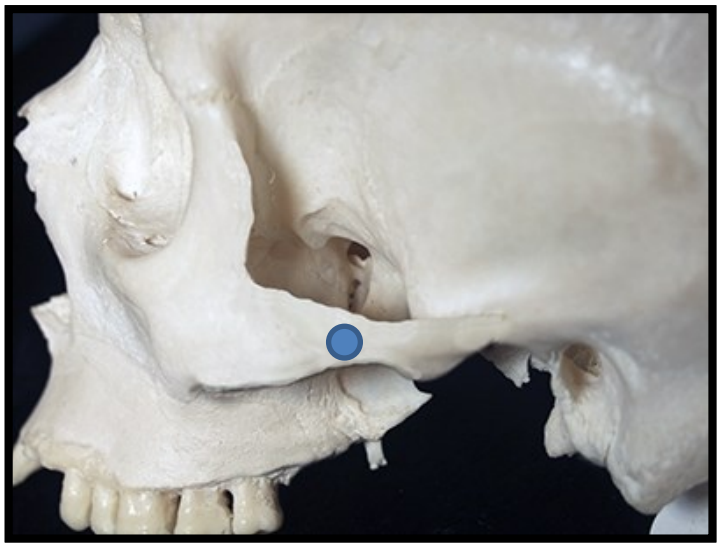


Figure 1.4 : Proposed location of zygoma wires.

Source : Melsen 2000 [2]

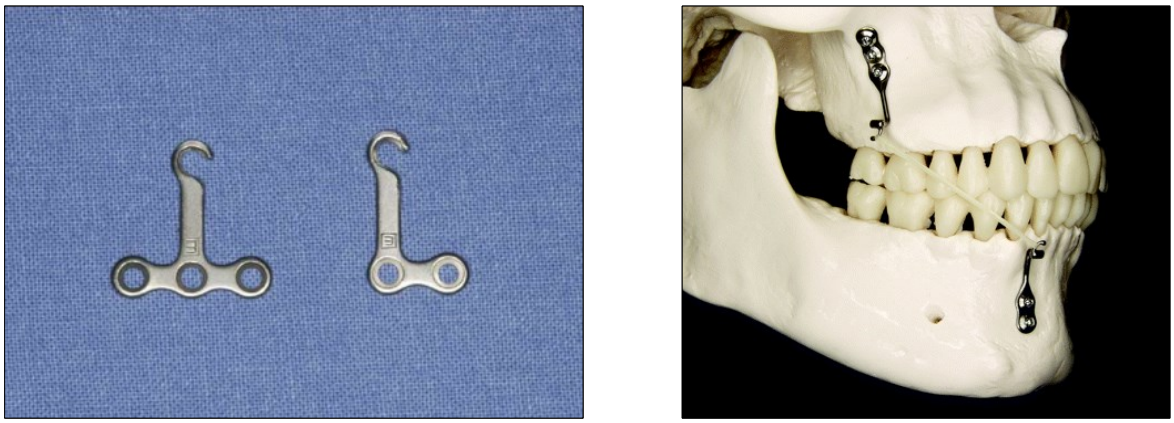


Figure 1.5 : Examples of miniplates, and their usage.

Source : De Clerke 2011 [4].

Furthermore, the removal process of this implant requires the use of a trephine to cut out the osseointegrated implant, a process that can be traumatic, and predispose the patient to further morbidity.

Such large size palatal implants are still used today [102], and advocated mainly for the reason that once osseointegration is achieved, it is difficult for the implant to fail during the treatment time, and anchorage is not lost.

In addition, the aforementioned study claim that overall treatment time may be reduced by as much as 5 months in comparison to a conventional dental anchorage control group. Further work is needed to investigate such claims, and it should be noted that popularity of large size palatal implants is declining due to the advent of orthodontic mini screw implants.

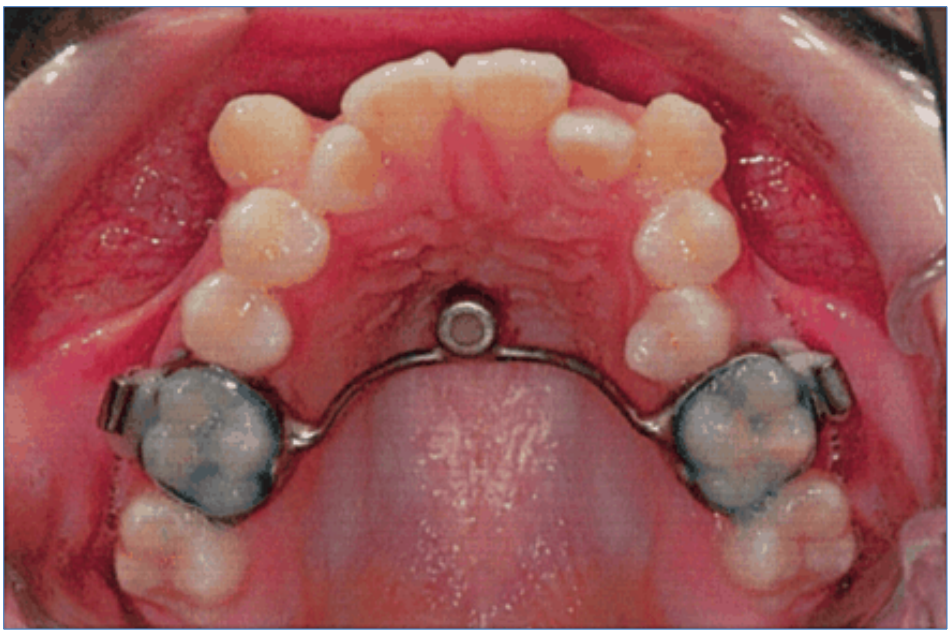


Figure 1.6 : Implant supported palatal arch

Source : Tinsley 2004 [5]

1.4 The orthodontic mini screw implant

Despite an improvement in the initial designs, many of the criteria for the ideal implant as described by Ismail and Johal [103] had still not been met. In particular, the increasing desire for early loading of implants used for orthodontic anchorage led to the rise of the other avenues [104].

Notably, in 1997, Kanomi [105] described a “mini-implant” specifically made for orthodontic use. This design was small (6mm in length and 1.2 mm in diameter) and easy to place or remove. The original design was developed from a mini-bone screw used for fixing bone plates. The author did not clarify how long the healing period would be to allow osseointegration, but did comment on the use of this implant for orthodontic space closure and molar distalization.

Furthermore, in 1998, Costa and Melsen [73] presented a similar screw with a bracket like head. This design has been developed further and today is known as the Aarhus implant [2]. The comparatively smaller size was the significant benefit of this generation of TAD allowed an increase in potential intraoral locations of these devices. Even interdentally between the roots of adjacent teeth.

In addition, this design allowed osseointegration to occur even in the presence of immediate orthodontic loading, and could easily be placed and removed when no longer required. It seemed that the initial criteria proposed by Ismail, and Johal [75] had been met. Several other mini-screw implants have been introduced, each presenting different designs and features, with clinical success and patient acceptance being described as good [98]. Some of the utilisation of OMSIs includes;

- Up righting tilted teeth [106]
- Retraction of individual of segments of teeth [107] [108] [109]
- Extrusion of individual or segments of teeth [110]
- Intrusion of individual or segments of teeth [111]
- Stabilization of teeth [112]
- To assist RME procedures [113] [114] [115] [116]

Interestingly, OMSIs have recently been proposed as foundations for restorations in areas where conventional bone volume limited the placement of a conventional osseointegrated implant [117] [118] [119] [120], or in cases where growth has not ceased and an aesthetic, non-invasive solution is required. Further studies are required in this field however, the concept is exciting.

Table 1.2 : Comparison of treatment with and without TADs.
Adapted from Reynders 2009 [121].

	Traditional orthodontic treatment	***Orthodontic treatment with TADs***
1. Anchorage	-Teeth, extra-oral structures (headgear)	-Teeth, TADs.
2. Stability of anchorage	-Position of anchor teeth is not stable during treatment	-Position is stable during treatment.
3. Number of anchor teeth	-In order to get sufficient anchorage, more teeth must be included.	-For direct anchorage teeth are not necessary. Minimal numbers of teeth are need for indirect anchorage.
4. Treatment efficiency	-In applying force on teeth, part of it is wasted due to periodontal amortization.	-Applying force on implant it is directly transferred to the moving part of orthodontic system.
5. Duration of treatment	-There is no reliable anchorage for transferring the desirable number of teeth at once, thus treatment time elongates.	-Stable anchorage enables transference of force to maximal teeth at once, thus treatment time shortens.
6. Patient cooperation	-Obligatory	-Minimal
7. Treatment acceptability	-Most treatment devices restrict patient's motions, and no not meet aesthetic requirements.	-Minimal discomfort for patient.
8. Professionals involved	-Orthodontist	-Orthodontist and oral surgeon
9. Side effects	-Undesirable change in anchor teeth position	-None significant to be reviewed in literature

Table 1.3 : Comparison of the various types of TADs and their use in orthodontic treatment.

	Prosthodontic implants	***Orthodontic implants***			
		Onplants	***Miniplates***	***Palatal implants***	***Mini-screw implants***
Anatomic sites for implantation.	-Alveolar processes of maxilla and mandible -Zygomatic processes of maxilla	-Median suture of the palate -Paramedian	-Any structure where sufficient cortical bone exits. -Commonly posterior molar / pre molar area	-Median suture of the palate -Paramedian	-Any structure where sufficient cortical bone exits.
Patient's age	-Not used until cessation of skeletal growth	-Used after ossification of median suture or the palate	None	-Used after ossification of median suture or the palate	None
Time of loading	-Loading after osseointegration complete (3 – 6 months)	-Loading after osseointegration complete (3 – 6 months)	Loading after healing	-Loading after osseointegration complete (3 – 6 months)	-Immediate loading
Type of surgery	-Usually flap and or bone surgery required	-Flap surgery required	-Flap surgery required	-Perforation of the mucosa needed -Perforation of the palatal bone needed	-Perforation of the mucosa needed
Postsurgical morbidity	-Variable dependent on surgical protocol -Usually swelling and or pain remain for a significant period (1-2 weeks)	-Usually swelling and or pain remain for a 1 week	-Usually swelling and or pain remain for a 1 week	-Minimum patient discomfort	-Minimum patient discomfort
Usage	-Mainly for restorative purposes -Can also be used for orthodontic anchorage.	- Orthodontic anchorage -Removed after treatment	- Orthodontic anchorage -Removed after treatment	- Orthodontic anchorage -Removed after treatment	- Orthodontic anchorage -Removed after treatment
Size	-3.0 – 6mm diameter -6mm – 18mm length.	-10mm diameter -2mm thickness	-2mm diameter -5mm (screw) length	-3mm diameter -4-5mm length	-1-3mm diameter -6-14mm length
Side effects	-High biological, financial, and time cost if an implant is to be lost. -Improper placement may lead to a failure of function and aesthetics.	-Propensity for Infection -Difficulty in removing	-Propensity for Infection -Surgical procedure required for removal	-Minimal	-Minimal

1.4.1 Definitions and classification

Due to such a varied development of anchorage devices, the literature is peppered with language such as mini-implants, mini-screws, micro-implants, orthodontic implants, intraoral extra dental anchorage systems [2], temporary anchorage devices [122], mini-plates and micro-screws. Although these are terms all used to describe various systems of skeletal anchorage, one would expect that such a hazy terminology may lead to confusion. Especially with the advent of other aspects of dental implantology.

For example, implants, and mini-implants make reference to systems which by Brånemark's definition imply that osseointegration occurs prior to loading. It has been shown that this is not a completely accurate method to describe the majority of systems skeletal anchorage in orthodontics today [123] [73] [124] [125] [2].

In 2005, Carano and Melsen [99] suggested that the word mini-implant should be applied both to palatal implants, mini-implants, mini-screws, and to micro-screws.

In addition, often, the prefixes mini- and micro- are concurrently used to describe implants or screws of the same dimension without any differentiation.

It must be noted however that the word micro pertains to extremely small dimensions, usually better observed with the optical assistance of a microscope [126]. Thus it has been advocated that the term mini-screw implant is more appropriate [127]. Since the arrival and popularisation of mini dental implants [128], and to avoid confusion with these counterparts more frequently used in restorative dentistry, this report will refer to mini-screw implants as orthodontic mini-screw implants (OMSIs).

1.4.2 Indirect and direct anchorage

Anchorage provision through OMSIs have further been classified to provide two different types of anchorage: direct and indirect [129]. Indirect anchorage involves the units be connected through bars or wires to the reactive unit. In this situation the OMSI stabilizes multiple teeth, which then serve as an anchor unit. The most common method of achieving indirect anchorage is by placing an OMSI in the midpalatal or retromolar regions, which are then linked to the natural teeth by means of a wire or other rigid fixation device, such as a transpalatal arch [130].

Indirect anchorage has also been suggested to reduce the peri-implant loading of the bone, and to reduce the risk of loosening OMSIs [131]. Holberg et al. [131] suggested in their FEM analysis, that the more anchor teeth were integrated into the anchoring block with indirect anchorage, the smaller was the peri-implant loading of the bone.

Direct anchorage refers to any situation in which forces that originate from the actual implant itself are used to augment anchorage.

An example would be a restored dental implant with an orthodontic bracket bonded to the restoration, or any mechanics attached to an OMSI [132]. Both methods provide a high level of stability and are equally useable in orthodontic tooth movement depending on the clinical situation they are utilised in.

It has been suggested that indirect anchorage is more versatile, and favourable for a number of reasons as previously outlined [129].

1.4.3 Types and properties

Between the currently available orthodontic mini-screw implants, significant differences exist in relation to their composition, size, and design. These may include; the materials used for fabrication, dimensions - diameter, the threaded portion, dimensions - length and the design of the head. Consequently, there are a number of commercially available orthodontic mini-screw implant systems available today, with the amount of manufacturers increasing rapidly.

As can be seen in the history and development of TADs, innovation remains an important in driving the succession of technology. However, it remains that science is often catching up to validate the application of such technology. The importance of evidence based medicine [133] is well documented and pivotal in order to ensure clinical decisions and protocols are supported with science.

Currently, the literature shows a lack of evidence in determining what surface characteristics the ideal OMSI possesses [51].

Table 1.4 : Table of currently available mini screw systems

Product	***Company***	***Website***	***Material***	
1. Ancor Pro System	Ortho Organisers, USA	www.OrthoOrganisers.com	Ti-6Al-4V ELI (Grade 23)	Ti machined (Anodised)
2. Aarhus Anchorage System	MEDICON eG, Germany ScanOrto A/S, Denmark	www.medicon.de	Ti-6al-4V ELI ASTM F136-02	Ti machined (Anodised)
3. AbsoAnchor System	Dentos, Korea	www.dentos.co.kr	Ti-6Al-4V ELI (Grade 23)	Ti machined surface
4. Benefit System	PSM Medical Solutions, Germany	www.psm.ms	Ti-6Al-4V ELI (Grade 23)	Ti machined surface
5. C - Implant	Dentium Inc., Korea	www.implantium.com www.cimplant.com	Commercially pure titanium	Sandblasted large grit and acid etched
6. Cizeta – Titanium miniscrew	Cizeta Surgical, Italy	www.cizetasurgical.it	Ti-6Al-4V ELI (Grade 23)	Ti machined surface
7. Dual Top Anchor System	Jeil medical, Korea Distributed by RMO Inc., USA	www.jeilmed.co.kr www.rmortho.com	Ti-6al-4V ELI (ASTM F 136) (Pure titanium)	Ti machined surface
8. IMTEC Mini Ortho Implant	IMTEC Corporation, USA	www.imtec.com www.3Munitek.com	Ti-6Al-4V ELI (Grade 23)	Ti machined surface
9. TOMAS Orthodontic Mini Anchorage Screw	Mondeal Medical Systems, Germany	www.mondeal.de	Ti-6Al-4V ELI (Grade 23)	Ti machined surface
10. Miniscrew Anchorage System (MAS)	Micerium S.p.A., Italy	www.micerium.it	Ti-6Al-4V ELI (Grade 23)	Ti machined surface
11. Orthoanchor K1 System	Dentsply Sankin Corporation, Japan	www.dentsply-sankin.com	Ti-6Al-4V ELI (Grade 23)	Ti machined surface
12. Orho EASY System	Foresta dent, Germany	www.forestadent.com	Ti-6Al-4V ELI (Grade 23)	Ti machined (Anodised)
13. ORLUS System	Ortholution, Korea	www.ortholution.com	Ti-6Al-4V ELI (Grade 23)	Ti machined Acid etched
14. Orthodontic Mini Implant (OMI)	Leone S.p.A., Italy	www.leone.it	Surgical stainless steel (ISO 5832/1)	Ti machined surface
15. Spider Screw Anchorage System	HDC, Italy	www.hde-italy.com	Commercially pure Ti	Ti machined surface
16. Temporary Mini Orthodontic Anchorage System TOMAS	Dentaurum, Germany	www.dentaurum.de	Ti-6Al-4V ELI (Grade 23)	Ti machined surface
17. Vector TAS System	Ormco, USA	www.ormco.com	Ti-6Al-4V ELI (Grade 23)	Ti machined (Anodised)

1.4.4 Biocompatibility

Historically, the usage of other materials in implant manufacture has also been attempted. For example; gold alloys, vitallium, cobolt-chromium, vitreous carbon, aluminium oxide ceramics, and nickel chromium-vanadium alloys [134].

Vitreous carbon implants showed a failure rate of 67 per cent [135] when undergoing orthodontic loading. In addition, Bioglass-coated[136] ceramic implants used for orthodontic anchorage were also unsuccessful. Despite the biocompatibility of these materials, these failures are believed to be attributed to an inability to osseointegrate.

Titanium of high medical grade (Type 4 or 5), has been shown to be both biocompatible, and capable of achieving osseointegration [137]. Consequently most systems available today are manufactured with medical type IV or type V titanium alloy. However due to an increase in demand, the cost for titanium is becoming more expensive [138]. Consequently, there has been movement to explore other material for manufacture of OMSIs ; i.e. Orthodontic Mini Implant (Leone S.p.A.), which is fabricated from stainless steel.

Stainless steel is one of the most frequently used oral surgical and orthopaedic implant materials because of a favourable combination of mechanical properties, biocompatibility, cost-effectiveness, and manufacturing ease [139] [140].

A large volume of literature is available on titanium alloy mini-implants and their types, properties, and loading behaviour [141] [125] [142]. In contrast, reports regarding the use of stainless steel mini-implants are scarce. Interestingly it has been demonstrated that stainless steel is more resistant to failure than titanium. However, its overall performance as material for OMSIs could be inferior to titanium [143]. Furthermore, there are variants of titanium implants including rough or smooth surfaces, and the presence or absence of an additional hydroxyapatite or titanium spray coating [144]. Interestingly, cellular response to grade 5 titanium has been shown to be superior [145], however, the overall conclusion remains that material type is less of a significant factor with respect to OMSI retention [146].

Recently a new design of biodegradable implant has been proposed [147]. It is designed to provide orthodontic anchorage similar to that of the orthodontic mini-screw, however instead of surgical removal, once redundant it is resorbed through foreign body reaction. The implant, which is made from a biodegradable polylactide, has shown adequate loading capacity for clinical application in orthodontics [148].

1.4.5 Osseointegration of OMSI

Roberts [149] used conventional, two stage titanium implants in the retro-molar region, to help reinforce anchorage whilst successfully closing first molar extraction sites in the mandible. After completion of the orthodontic treatment, the implants were removed using a trephine and histologically analysed. A high level of osseointegration was found to have been maintained, despite the orthodontic loading. In another study, Turley [150] used tantalum markers and bone labelling dyes in dogs to illustrate the stability of two stage implants in cases of orthodontic or orthopaedic traction. Whilst many of the original studies were completed with sources of skeletal anchorage that had osseointegrated, there is a question whether a full implant osseointegration is needed in orthodontics when relatively small forces are applied.

In a review of studies exploring implantable orthodontic anchorage, Favero [151] postulated;

"Some studies have shown that implants loaded early on, although not presenting intimate bone-to-bone contact [osseointegration] because of the formation of a pseudo-peri-implant fibrous ligament appeared to be sufficiently stable and capable of sustaining the function of anchorage with normal orthodontic forces. Did these represent failures, because osseointegration did not occur, or successes, because the anchorage was achieved anyway?"

Currently it is believed that complete osseointegration of screws used in OMSI is a disadvantage as it complicates the removal process [152]. Although surface characteristics for mini-implants include etched and sandblasted varieties, many of these devices are manufactured with a smooth surface, minimizing the adaptation of biological tissues. Furthermore, Chaddad [144] showed that surface characteristics between machined titanium, and sandblasted acid etched surfaces did not affect the survival of immediately loaded OSMI.

Interestingly, one manufacturer (C-Implant), continues to manufacture and promote a sandblasted acid etched surface. There has been some evidence to suggest that this type of surface treatment is more conducive to osteocytes [153] [154] [154]. However, whilst OMSIs are considered to achieve their retention purely mechanically, the notion of osseointegration should not be dismissed entirely [155]. OMSI with varying surfaces to encourage osseointegration have been used successfully [156]. In addition, limited osseointegration has been demonstrated in both *in-vitro* and *in-vivo* studies. [157] [158]

1.4.6 Thread and body design

The thread and body of the various implant systems can either be conical (eg. Aarhus Anchorage System, the AbsoAnchor System). Alternatively, it may be parallel, with tapered ends (eg. Orthodontic Mini Implant). Both designs have been proven to have close approximation with bone after insertion [159] [160] [144], as well as effective [161] at successfully maintaining orthodontic loads. However, Wilmes [162] described clinically significant correlation between the two, with conical threaded designs achieving superior primary stability in comparison to the cylindrical design. This is a finding corroborated by others [163] [164] [165].

It has further been shown that conical fixtures are more likely to fail in the mandible than conical fixtures in the maxilla due to the variation in type and quality of the bone [166]. This is thought to be as a result of increased torque concentration as a result of geometric concentration, and the denser cortical bone.

Based on its radiographic appearance and the resistance at drilling, bone quality has been classified in four categories:

- **Type 1**; bone in which almost the entire bone is composed of homogenous compact bone.

- **Type 2**; bone in which a thick layer of compact bone surrounds a core of trabecular bone;

- **Type 3**; bone in which a thin layer of cortical bone surrounds a core of trabecular bone;

- **Type 4**; bone characterized by a thin layer of cortical bone surrounding a core of low density trabecular bone of poor strength.

These differences in bone quality can be associated with different areas of anatomy in the upper and lower jaw. The mandible is generally more densely corticated than the maxilla and both jaws tend to decrease in their cortical thickness and increase in their trabecular porosity as one moves from the anterior, towards the posterior. This poses significant implications when placing any type of dental implant.

It has been shown by the above authors that the tapering nature of these fixtures concentrates forces and thus leads to high levels of torque. Highly increased torque when placing mini-implants has been shown to lead to higher failure rates [167] [168] [169]. This increased torque may lead to micro damage of the bone and surrounding tissues, including necrosis and local ischemia [170] [171] [167]. Perhaps conical type fixtures may be considered in the maxilla, as the generally lower quality of this bone reduces the likelihood of force concentration – a factor that has been echoed in the literature.

Furthermore, thread design is also a consideration that affects stability and torque when placing or removing OSMI. Kim et al. [172] found that dual-thread designs generally show a low insertion torque however higher removal torque than cylindrical and tapered designs. Perhaps dual threaded designs may be incorporated into conical OMSI design to reduce the high levels of insertion torque discussed in the literature.

When attempting to place OMSIs in densely corticated regions of the mouth two delivery systems have been proposed; self-drilling and self-tapping. The self-drilling OSMI is designed with a piercing tip to penetrate the overlying tissues and bone. It is attached to a handpiece or manual screwdriver, and drilled directly through the soft tissues and bone into its final and desired location.

The self-drilling system is generally less invasive with no surgical flap being needed, consequently this results in less post-operative discomfort and patient acceptability [98]. It has also been suggested that this leads to faster healing and a decreased risk for infection [173]. In addition, it has been illustrated that self-drilling screws offer more osseous resistance than self-tapping screws [174]. However, the self-drilling systems may cause increased pressure and damage to the overlying, and deep tissues the effects of which have been discussed.

In addition, there has been speculation that soft tissue fragments and bacteria from the oral cavity can become transported through the flutes of the OSMI into the bone. To the author's knowledge, the effects of this have not been described in the literature.

The self-tapping mini-implant does not possess a piercing end, but rather a flat or dome shaped end. A surgical flap is usually raised to visualize the bone so that a pilot hole may be drilled with a handpiece to guide the OMSI. The pilot hole may be partially or completely through the bone depending on the amount of resistance. The OMSI is then placed into the pilot hole and screwed into its final location.

Self-tapping systems offer greater control in the location of OMSIs, however due to the soft tissue trauma, a longer healing time and postoperative discomfort may be expected. In addition, there is a risk that the more aggressive rotary instrumentation required to pre drill these sites, despite their non-cutting tips may cause propensity to damage dental apices.

It has also been suggested recently that improper technique, and or selection of pre-drilling the site may lead to reduced primary stability for the OMSI [175]. In his study, Fritz et al[176] omitted pilot holes in the maxilla and, when appropriate, in the mandible, since they could impair the primary stability of the implants. For self-tapping OMSI, the diameter and the length of the implant has been recommended to be 0.2 to 0.5 mm larger than the width and the depth of the bone hole for optimal placement torque[177]. This was the most important factor for successful immediate and early loading [178].

Furthermore, Motoyoshi et al. [179] found that insertion torques of 5 – 10 Ncm was adequate when placing OMSI in both the mandible and maxilla. In addition, Otani et al [180] reported that failure rate was particularly high for self-drilling mini-implants (diameter 1.6 mm, length 6.0 mm) placed into adult female maxillary buccal alveolar bone with a torque of either less than 5.0 Ncm or more than 16.3 Ncm.

Although 5 – 10 Ncm is currently accepted as the “gold standard” with respect to insertion torque of OMSI, conflicting evidence can also be found in the literature [144] [181] [182]. In addition, a recent systematic review on the topic of insertion torque regarding OMSI has been deemed inconclusive [178].

Wawrzinek et al. [167] also examined the cortical bone surrounding mini-implants using SEM, and found microstructural damage due to over-tightening via deep insertion of orthodontic mini-implants, but they did not examine the histology of the surrounding cortical bone. In the mandibular model used in this study, cracks in the surrounding cortical bone were observed in the self-drilling group and in the pre-drilling group that used pilot holes 0.7, 0.8, 0.9, and 1.0 mm in diameter. In the maxillary model, no cracks were observed in the surrounding cortical bone in any of the groups. In another similar study, Tachibana et al. [183] also found that self-drilling in the maxilla without a pilot drill was acceptable, whilst in the mandible; a pilot hole of 1.0mm in diameter is recommended. This was corroborated by Uemura et al who came to similar findings [184]. Overheating of the pilot drill causing bone damage, might also contribute negatively, thus copious irrigation with saline when drilling is recommended. Stress concentration, is a vital factor when torqueing OMSI. Due to their smaller dimensions, it is important to appreciate the limitations of the materials. For example, thread stripping and implant fracture can occur if maximal torques are reached [179] [185].

1.4.7 Bone quality and OMSI stability

Bone quality, and biology are the underpinning factors regarding orthodontic treatment, and also treatment with OSMI.

With conventional dental implants, it is not primarily the ratio of cortical bone to trabecular bone that influences implant stability as much as the absolute amount of dense cortical bone. Cortical bone has a higher modulus of elasticity than trabecular bone, is stronger and more resistant to deformation. Consequently, it will bear more loads in clinical situations than trabecular bone. What applies to traditional dental implants also applies to orthodontic mini-implants: thicker cortical bone provides greater primary stability. In addition, there have been various systems proposed to describe bone quality in dental implantology[166].

In the case of conventional dental implantology osseointegration is a prerequisite for functional loading of the implant. However, when considering OMSIs, it is not necessary to wait for bone healing and osseointegration to occur because a OMSI gains its primary stability from mechanical retention and can support immediate orthodontic loads [124].

The maximum load for a non-integrated implant has been shown to be proportional to the surface area of the implant in contact with the surrounding bone [186].

Higher failure rates of OMSIs have been experienced by clinicians where they were placed in patients with reduced cortical bone thickness. Miyawaki[187] reported that the failure rate of OMSIs was correlated with the mandibular plane angle, but other factors, such as screw diameter and that peri-implant inflammation, may also contribute to failure[187] [177]. Furthermore, it has been proposed that in sites with extremely dense bone such as the mandible, the median parts of the upper alveolar ridge and the hard palate, the recipient site should be predrilled carefully, and with an appropriate sized drill to avoid unwanted risks such as implant fracture, and poor angulation.

According to *Costa*[188] and *Miyawaki*[187], cortical bone quality and quantity are major factors associated with the primary stability of OMSI, most likely because it is achieved by mechanical retention rather than osseointegration. Wilmes et al[189] found that cortical bone thickness has a strong effect on the primary stability of OMSIs. Placement torque and pull-out strength of OMSIs have also been correlated with cortical bone thickness [190].

Thicker cortical bone has been reported for the mandible than the maxilla, with bone in the mandibular buccal region reported to be thicker than bone in the mandibular lingual region. This has been shown to range from between 1.09 to 2.12 mm in the maxilla and 1.59 to 3.03 mm in the mandible.

Furthermore, at the same site in respective jaws, moving in an apical direction, cortical bone became thicker. There also appears to be differences in each jaw with the thickest cortical bone in the molar region, followed by the premolar and incisor regions, respectively. This has been hypothesised to reflect the loading characteristics of the respective teeth. Molars tend to accept the highest force, and hence have maximum osseous support – hence thicker cortical bone.

Thicker cortical bone in the buccal region of the mandible might be explained biomechanically. Whereas the mandible is under torsional and bending strains, the maxilla is generally subjected to more compressive forces. It has also been demonstrated that regions that experience higher strain during function develop thicker cortical bones [191].

Baumgaertel [192] studied palatal bone depth with respect to orthodontic implant placement in adults and found that cortical bone depth decreases as we move from anterior to posterior. He found the mid palatal suture to be a good site for implant placement, with the area in line with the first and second premolars being the best. In addition to bone volume diminishing as one proceeds more posteriorly, soft tissue also thickens, and the presence of significant blood vessels and nerves increases risk of implant placement.

This notion was echoed by Laursen and Melsen in their recent cadaver study using micro-CT [193]. These authors also extrapolated that changing the fixture insertion angle to 45 degrees will generally enhance implant stability, but may increase perforation to the maxillary sinus when placing these fixtures in the maxilla. Similar findings have been reported by other authors [194]

Baumgaertel [195] also suggested that the posterior palate should by encouraged as a placement site for OMSIs. In a recent symposium, others have also advocated the palate as a more favourable site for OMSI implantation as it provides more favourable characteristics including increased bone volume in comparison to other sites. Moreover, by inserting into the palate, any orthodontic tooth movement is not limited by the placement of the OMSI – a factor inter-radicular placement is affected by.

The localised anatomy, and bone morphology is vital in the placement of OMSI. Due to the great deal of anatomical variation in individuals, volumetric analysis is suggested to investigate cortical bone thickness prior to making any clinical decision. Interestingly, numerous studies have concluded that available bone is more voluminous than what is apparent in radiographic survey including CBCT and cephalometric analysis, allowing for a “safety distance” to be pre-built into clinical decisions on implant length.

Interestingly, Ansari et al. [196], demonstrated that arch form also has a relationship on cortical bone thickness with square arch form having statistically significant thickness in cortical bone. This study also suggested that patients with ovoid arch form may require implants with increased features promoting primary stability as these arches suggested deficient cortical bone.

Other variations’ between sexes have suggested that females have lesser volume of cortical bone in the anterior buccal region than males, although other studies have not correlated differences in gender and cortical bone volume.

1.4.8 Length of implants

In addition, OMSI are available in different lengths and diameters to facilitate placement at various anatomical sites. Although the work of Costa et al [188] has shown that OMSI of length 4 to 6 mm are safe in most regions, individual variation necessitates volumetric analysis and evaluation of bone depth in all patients to minimise unwanted effects. Most OMSIs are available in length from 4.0 to 12.0 mm [197] [198]. However in some cases increased length is required. There have been reports of OMSI of length 14.0mm[198] and even 21.0 mm[199] being used.

Miyawaki et al[187] has reported that there is no association in the length of the screw with its stability if the screw is at least 5 mm long. In addition, Fritz et al. [176] has suggested that 4 mm long screws offer adequate stability when compared with 6 mm and 8 mm screws. Also Cheng et al.[200] and Kanomi[105] argue that smaller and rather short implants serve the purpose as well. However due to the reduction in length, the primary stability is reduced which then has to be recovered by the stabilising edge and threads [201].

Interestingly, another study by Chen[202] has found that the success rate of OMSI rose from 72% to 90% by using 8-mm instead of 6-mm long screws. Other studies have also corroborated this showing higher success rates with additional length at the same diameter. [203] [197] [202] [198] [204]

When selecting length of an OMSI, it must be noted that due to varying transmucosal thicknesses, one must be careful to select a length that will ensure most of the implant is anchored in bone, rather than soft tissue. The latter will obviously have poor implications for the success of the implant [205].

Tseng et al[198] also found that length of the OMSI was an important risk factor, correlating it to success. These authors emphasized that the actual depth of insertion of impant was more important than its length, the recommended length being at least 6 mm - Possibly to account for the transmucosal thickness. This is in accordance with the general findings in the field of dental implantology, where the shorter and smaller diameter implants have led to lower survival rates than their counterparts [206].

Bicortical OMSI have also been suggested by some authors [207] [208] [6]. This involves placing the OMSI across the entire width of the alveolus, usually engaging both cortices. Brettin et al[6] showed that such a system could provide a force system with superior force resistance and stability (anchorage) compared with monocortical placement. They found that deflection force values were significantly greater for bicortical than for monocortical screws, concluding that bicortical OMSI placement provides superior anchorage resistance, reduced cortical bone stress, and superior stability than monocortical placement.

In addition, it has also been suggested that since vertical placement does not impact mean anchorage force resistance, placement of either a monocortical or a bicortical screw in a more coronal position within attached gingiva limits the possibility of peri-implant inflammation[9,125].

Furthermore, as well as providing an environment more receptive of biological compatibility, coronal placement of screws may also be biomechanically advantageous because they are closer to the centre of resistance of the teeth [209].

However, despite their inherent advantages in terms of maximal anchorage and stability, the major disadvantage is the surrounding anatomic limitations.

Placement of such devices is generally riskier with greater propensity to injure structures. Furthermore, manoeuvring such long screws inside the oral cavity may be difficult in the posterior areas, where cheek retraction is a limitation. Perhaps one instance where bicortical screws may be utilised is where a conventional diameter monocortical screw has failed, and a larger diameter monocortical screw would jeopardise adjacent structures[209].

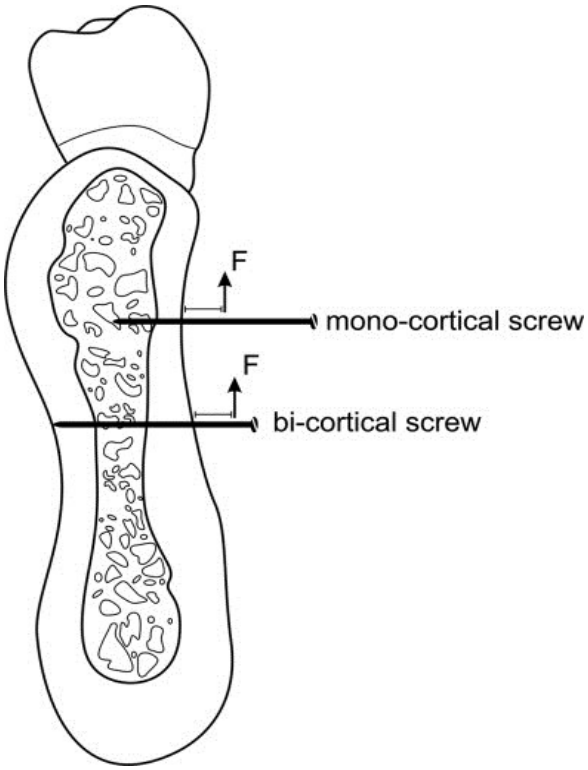


Figure 1.7 : Orthogonal view of monocortical and bicortical OMSI placement in bone specimen.

Source Brettin et al. 2008 [6]

1.4.9 Diameter of implants

Diameter of the orthodontic mini-screw implants is also an important factor affecting their placement. For example, a narrow implant may be more easily and safely inserted to sites with root proximity without the risk of root contact. On the other hand, a narrower diameter combined with shearing and torsional stresses during the placement and removal of implants may lead to an increased risk in fractures. This has been especially noted in OMSI with diameters of less than 1.3 mm[209 210] .

Miyawaki et al[187] reveals that implants of less than 1 mm in diameter are the least reliable. They reported that 1 year success rate of implants with a 1.5 and 2.3 mm diameter was significantly higher than that of implants with diameters of 1 mm. In addition, it was found that patients with a high mandibular plane angle displayed a significantly worse success rate than those with an average or low angle. This has been attributed to the thinner cortical bone in molar region. Consequently, they have suggested that other features to maximise primary stability should be employed when placing OMSI in patients with vertical facial growth.

Berens et al[211] also found similarly low success rates with narrow diameter OMSIs, as did Carano et al[99]. Interestingly, Ohmae et al[212] illustrated that OSMIs of 1 mm in diameter and 4 mm in length placed in the mandibular third premolar region of beagle dogs were able to sustain an intrusive force of 1.5 N for 12 to 18 weeks. This differing finding has been echoed by Park [213] who reported implants with a diameter of 2.0 mm had the lowest success rate when compared with their smaller diameter counterparts. Kuroda et al[98] further confirmed that small diameter implants had a higher success rate than larger ones.

Although controversy currently exists as to which is the best diameter for OMSIs, two separate, recent systematic reviews of the literature suggest that the ideal OMSI diameter should be between 1.3 – 2.3 mm [121] [177]. It should also be noted that one such review concluded that implants with a diameter of 2 mm cannot be considered safe for placement in the posterior inter-radicular spaces of the maxilla, except between the first molar and the second premolar on the palatal side, and between the canine and the first premolar [214]. Most commercially available OMSIs are available in a minimum diameter of 1.5 mm

1.4.10 Head design

Although their inception was originally inspired through conventional bone screws, OMSIs differ from conventional bone screws because of a dual head i.e. the head has an additional feature designed for orthodontic treatment. The most frequent is the button like design with a sphere or a double sphere-like shape or a hexagonal shape. This design commonly has a hole through the head or the neck of the screw, usually 0.8 mm in diameter, and is commonly used for direct anchorage. A bracket-like design is also available, which can be used for either direct or indirect anchorage. Another permutation of the head design comes from the TOMAS mini-screw implant which offers a hook design.

The head of the OMSI is exposed to the oral cavity, and is connected to the underlying threaded portion of the screw via a transmucosal collar or neck. The transmucosal collar can vary in length depending on the requirements of the operator. It should be noted that most OMSIs feature a similar surface on the head, transmucosal collar, and thread.

Figure 1.8 : Some of the various head designs available with OSMI systems.

Source : www. Jeilmed.co.kr

1.4.11 Diagnostic imaging for OMSIs

Diagnosis and treatment planning remains one of the corner stones for sound orthodontic treatment. With the advent of implantology in the field of orthodontics, there is a need for diagnostic imaging not conventionally sought. Perhaps the greatest risk posed by OMSIs is the safe placement of these devices without irreversible damage to vital structures[215 216 217].

Orthopantomograms, cephalometrics and even intraoral radiography have served clinicians well historically[218]. However, with the development of technology, one may realize that such conventional methods do not often accurately depict the anatomical relationship of structures, as they only provide two-dimensional views. Consequently, without the full picture, clinicians are working unaware of any limitations or dangers in the field, especially with surgical procedures. Furthermore, as radiographic artifact and magnification may be introduced, their results of these radiographic methods must be interpreted with caution [219 220].

When placing OMSIs, the limited dimensions of these devices allow them to be placed between adjacent tooth apices. Consequently, the level of accuracy and precision in determining the unseen anatomy of these regions is crucial if one is to avoid damage during OMSI placement.

In addition, since factors such as cortical bone thicknesses have been implicated in success[221] and thus locating a suitable site for OMSI placement, clinicians are searching for more data in their diagnostic imaging. This calls for more advanced technology.

Whilst computed tomography (CT), can provide accurate measurements of small areas in bone[222,223 224 225] , traditional CT imaging is expensive, and has a concerning relative radiation dose. The advent of cone beam CT (CBCT) works on a similar concept of CT imaging, and maintains good image resolution and accuracy[226 227 228] with decreased patient exposure and expense[229].

Interestingly, the work of Motoyoshi[191] has shown that knowledge of the thicknesses of cortical bone throughout the jaws is directly linked to the success of mini-implants.

In addition, if using their protocol, Liu et al[230] have shown that as well as helping to localize OSMI, CBCT can also be used to manufacture surgical guides which can accurately guide the safe placement of OMSI. This has been a finding echoed by others in the literature [231 232].

Interestingly, in a recent article, Jung and Wehrbein's [233] work showed that out of 101 patients, when placing palatal TADs, CT or CBCT analysis showed that 98% had sufficient osseous volume to accommodate such a fixture. They pose the question that: Is volumetric analysis necessary in such cases? The aforementioned author's recommend that initial screening be completed via standard orthodontic radiographs such as a lateral cephalograms, and CBCT be reserved for rare exceptional cases.

1.5 Clinical factors regarding OMSI

Specific recommended procedures for inserting orthodontic mini-screw implants are often available in the product documentation. However, some basic guidelines follow:

1. Informed consent must be given prior the patient to proceed with treatment.

2. Adequate volumetric analysis, and or conventional radiographic analysis must be completed to ensure good planning protocol.

3. A small amount of local anaesthesia is suggested and it is advocated not to achieve profound anaesthesia of the teeth but only of the soft tissue [204.] This allows detection of any root proximity errors by a nociceptive alert.

4. If the system is not a self-tapping mini-screw, drilling a pilot hole is necessary. Pilot drilling should be done in a surgical environment, and if necessary, by an oral surgeon. Firstly, soft tissue from the site of the placement is either incised or removed using a soft tissue punch. Thereafter, a pilot hole is drilled using a drill rotating no more than 1000 rpm. The pilot drill is usually 0.2 to 0.3 mm thinner than the mini-screw implant[53]. The mini-screw implant is then screwed in place by using an appropriate screwdriver.

5. In case of self-drilling mini-screw implants, no incision or soft tissue removal is necessary. Infection control is similar to that for an extraction. After selecting the appropriate site, the mini-screw implant, and the corresponding site of placement, it is inserted in place. This may be either with a hand driver or rotary placement.

Surgery-related factors affecting the success of OMSI placement include; experience of the surgeon, sterilization, flap or flapless surgery, self-tapping or self-drilling technique, pilot hole preparation in the cortex only or for the entire screw depth, diameter of the pilot hole, cooling technique, drill speed and pressure, direction of placement, steady or wiggling placement procedures, monocortical vs bicortical anchorage, and placement torque.

Excessive surgical trauma and thermal injury can lead to osteonecrosis and fibrous encapsulation of the implant [234] [235]. Interestingly, it has been suggested that failure rates can probably be reduced with increasing clinical experience [176]. This is particularly relevant in the field of orthodontics where operators may not have had exposure to surgical procedures for protracted period of time.

Angle of implant insertion was another factor that has been found to affect implant stability. Lim et al, Kyung et al, Carano et al. and Wilmes[236] [237] have all found that proposing a degree of angulation during insertion increases implant to bone contact, and thus primarily stability. Wilmes[236] [237] has advised this angle to be around 60° to 70°. However, whilst primary stability is increased, care must also be taken to avoid severe angulation.

Wilmes[236] [237] also advised against routine angulation of OSMI as there is a risk of slippage of the mini-implant at its first contact with bone. In addition, obliquely inserted mini-implants may expose a greater lever arm if forces are applied with the risk of higher failure rates [186].

Thus whilst angulation of OMSIs may be useful in areas of poor bone quality such as the maxilla, care should be taken to adopt such techniques routinely in the mandible. Note that another reason to consider angulation of OMSIs may be in areas where the apical anatomy needs to be manoeuvred.

Furthermore, it has been proposed that the soft tissues also play a significant role in success or failure for OMSI [238]. As with conventional dental implantology, surgical placement in a non-keratinised site compromises the peri-implant interface by reducing the defences to bacterial ingress and potentiating an inflammatory response eventually leading to osseous destruction and the loss of the implant.

A similar notion applies to OMSI with surgical placement in keratinized gingiva thought to reduce the development of hypertrophic tissues and inflammation [239]. A relationship between success and the character of the soft tissues has been proposed as evidenced by the literature [210] [198] .

1.5.1 Loading and anchorage considerations

Despite the early designs of skeletal anchorage focussing on a sufficient osseointegration period, part of the popularization of OMSI is that they may be loaded immediately. It must be noted however that only light forces are recommended early on.

The work of Miyawaki et al. Has shown that force can be applied straight away to orthodontic mini-screw implants if the force is under 2N[187 240]. Interestingly, even with light force, Zhao[241] et al did not recommend immediate loading, rather a delayed 3 week period preferring some consolidation prior loading to ensure better success. A similar notion has been echoed in the literature with a delayed loading protocol avoiding the initial 3-4 week decrease in OMSI stability [242 243].

However, in their systematic review, Chen et al. [177] showed that most mini implants can withstand 100 to 200 g of horizontal early or immediate loading successfully; that is enough to sustain the various orthodontic tooth movements.

Another factor that needs to be considered is whether or not OMSI actually provide true absolute anchorage. Liou et al. [124] found that mini-screw implants might move according to orthodontic loading in some patients, and it is therefore advised to allow some manner of safety clearance to prevent proximity error to apices [244]. This can lead also lead to anchorage loss. Thus if a clinician is to utilize OMSI in their armamentarium, one must be vigilant about maintaining good success rates and minimising failure [245].

1.5.2 Clinical factors for OMSI survival

Selecting an adequate implant site is a crucial factor in the overall success. Numerous factors have been identified in determining an adequate site for implantation.

Although most of the TADs as described are only required as temporary anchorage, implying their eventual removal, it is vital to consider that good healing, and healthy tissues are a prerequisite to success with this treatment modality. Consequently all of the general health requirements that apply to conventional dental implant treatment planning pertain to orthodontic mini-screw implants, and similar systems. These include;

- Age
- Smoking status [246] [247] [248]
- Diabetes and Immune status [249] [200] [250]
- Bone quality and site selection.

It should be noted that all of the absolute contraindications for OSMI treatment pertain to systemic and localised bone health. These include history of bisphosphonate therapy, hypersensitivity, titanium allergies, metabolic bone disorders, bone pathologies, poor bone healing, cardiovascular disease, psychosomatic disease, uncontrolled periodontitis, undergoing radiation therapy, or localized active infection.

Local risk factors

Obviously, prior to perforating the cortical bone in any region of the oral cavity, appreciation needs to be made for the local anatomy. Areas where damage to adjacent structures such as roots, veins, arteries, nerves, the maxillary sinus and the nasal cavity should be avoided. Prior to surgical intervention, adequate volumetric analysis may help in avoiding unwanted anatomical relationships. Anatomical locations that have been suggested for the placement of OMSIs include [251];

The treatment objective perhaps is the most important with consideration needing to be made in why the OMSI is being placed, how long it will remain in situ and the biomechanics it will underpin. The biomechanics are advised to be simple, with consideration given to future tooth movement to avoid any interference with the implant. The implant site should also provide sufficient attached gingiva to prevent patient discomfort, tissue overgrowth, and micro jiggling that can lead to long-term implant failure [177].

Sufficient inter-radicular distance should also be allowed to ensure that no damage is inflicted to adjacent tooth roots [214] [252]. Although the contemporary literature tells us that it is difficult to completely perforate an apex with a self-drilling screw [215].

In the event of more likely superficial damage to an apex, some studies have shown despite minor injury there is good propensity for repair [215] [217] [253]. However, root proximity has been associated with increased implant failure [216].

Often, the ideal implant site may be obstructed by root proximity. Consequently, roots may need to be up righted and aligned to facilitate successful implant placement.

Alsamak et. al [254]. in their systematic review concluded that most favourable areas for OMSI insertion in the maxilla are proposed between the first and second molars buccally and palatally. The best area in the mandible is also between the first and second molars, both buccally and lingually. In the palate, the paramedian area 3 to 6 mm posterior to and 2 to 9 mm lateral to the incisive foramen was identified as the best site for OMSI.

Considering all of these factors, recently there has been a trend moving away from placement in inter-radicular areas. Instead, palatal placement of orthodontic mini screws attached to some type of hybrid prosthesis have become popular as they allow less anatomical risk, and increased versatility. In addition, due to placement in more favourable mucosa, anecdotal evidence suggests reduced failure, and complications. This type of placement has been suggested by Cope [255], Baumgaertel [256] and Wilmes [257].

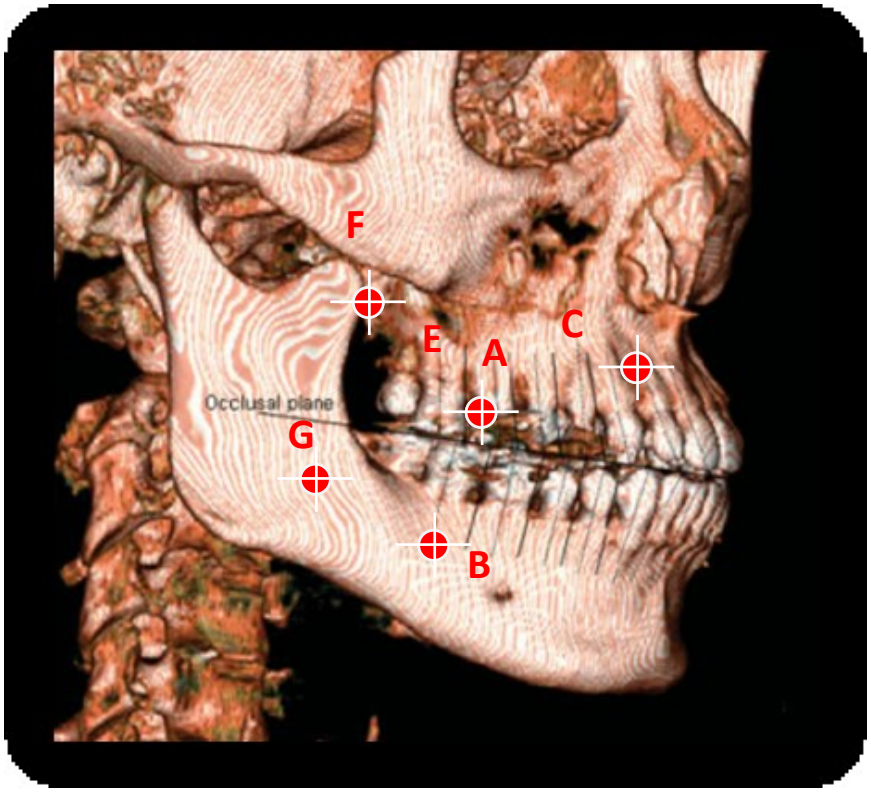


Figure 1.9 : Potential sites for placement of OMSI

A - Maxillary alveolar bone, B - Mandibular alveolar bone,
C - Palatal bone, D - Infra-zygomatic crest, E - Maxillary tuberosity,
F - Inferior ridge of priform aperture, G - Retro-molar region.

1.5.3 Complications of OMSI

All practitioners are obliged to fulfil the Hipporatic notion of “do no harm”. Recognition of these principles requires the appreciation that with any treatment risks and benefits exist. There are a number of complications that may be associated with orthodontic mini screw implants [258].

Perhaps the most common complication is failure of the device. It has been reported that currently, approximately 10-30% of orthodontic mini-implants fail [211 259 200 176 187 260 94].

Although failure may be due to a host of reasons, one of the identified factors may be peri implantitis. Peri implantitis remains a pertinent factor for the failure of osseointegrated endosteal dental implants [261 262 263]. The literature relating to peri implantitis of OMSI is relatively low [264 265].

Some manufacturers offer mini-implants of significantly larger diameter that can be placed immediately in the site of a failed implant. However, extreme caution must be used to prevent damage of the adjacent roots [266 267]. A healing time of 2 to 3 months before placing a new implant of the same diameter in the same location is recommended to allow for the bone to fill in.

Perhaps the most morbid outcome of OMSI failure may be aspiration if the implant becomes completely dislodged from the appliance. This may lead to pneumonia, and or surgical intervention to remove the foreign body. However, it should be noted that such complication can occur with any similar orthodontic appliance such as a bracket or other elements of the dental armamentarium.

Interestingly, there has been one such report of alveolar bone exostoses subsequent to OMSI placement [268]. Whilst this may not be significant or representative of the overall sequelae in relation to OMSI placement, further investigation is needed in such potential outcomes.

Some authors have explored the notion of subsequent bacteraemia after placement, and or removal of OMSI where microbes originating in the oral environment may become systemically involved [269]. Although the mentioned study did not detect any evidence of bacteria subsequent to OMSI placement systemically, further work is required to investigate this idea in more detail.

Whilst care needs to be taken during the planning process, damage to adjacent structures can occur even though orthodontic mini-implants and pilot drills are specifically designed to not cut into roots.

Therefore, damage of the root proper is rare, but it is possible to damage the structures of the periodontal ligament. The minimal space requirement between roots is 0.5 mm mesial and distal to the implant, or 1 mm more than the implant diameter.

Whilst other structures such as the neurovascular bundle and maxillary sinuses are also at risk, their involvement in OMSI treatment is often uneventful.

As with many aspects of cutting edge technology, perhaps cost may be an implication for patients as protocol is still evolving on how and where such clinical procedures fit into the array of tools that we offer

1.5.4 Current success and failure rates

As discussed, despite the many advantages that they present, the clinical behaviour of orthodontic mini-screw implants is still unclear and unpredictable. Although variable, the current, failure rates of OMSI has been described in the literature as between 10%-30% [211 259 200 176 187 260 94]. Such data suggests that this is still not satisfactory. As discussed prior, retention of OMSI is reliant on various influencing factors. These may be;

- Implant type, the implant dimensions[110 177]
- Implant surface characteristics
- Insertion angle [236]
- Pilot drilling hole size [270 175]
- Insertion torque [179 178]
- Force magnitude [200 186 260]
- Anatomic location [260]
- Primary stability of the implant [271 179]
- General health status of the patient.
- Soft tissue characteristics [200]
- Inflammation of the peri-implant area (Peri implantitis) [9 272]
- Possible root proximity [215-217]

In addition to these factors, general surgical principles need to be applied to maximise clinical success.

Despite the research that has already been done in this field, further work is needed as an ideal method for achieving stable implants in the initial integration stage has not yet been developed. Further investigation is needed and studies seem not to have drawn specific conclusions yet. Melsen states that; “Their success rate is depended on the system used, the operator (technique and experience), and the patient (multiple factors).”

Patient related factors	Implant related factors	Management related factors
-Age -Sex -Type of malocclusion -Thickness and kind of mucosa -Features of the bone -Thickness of the cortical bone -Location in relation to roots -Soft tissue inflammation -Hygienic care -Smoking habit	-Type of TAD -Length of TAD -Diameter of TAD	-Time of loading -Type of movement -Clinician

Table 1.5 : Summary of factors associated with TAD failure or success.
Source: Adapted from Dalessandri et al 2014 [272]

1.6 Biofilm formation on dental implants

Exposure of an osseointegrated implant in the oral cavity inevitably leads to biofilm formation. The first stage is for an acquired pellicle to develop on the implant surface through selective adsorption of the environmental macromolecules such as alpha-amylase and serum albumin [273]. This pellicle is derived from components in the saliva, as well as bacterial and host tissue products. It acts as a substrate for bacterial colonization, which occurs as early as 30 minutes after implant exposure in the oral cavity [274].

Biofilm represents an organized structure in which microorganisms interact metabolically as a community [275]. Biofilm formation around implants occurs in a similar way as teeth. After formation of the acquired pellicle, bacterial attachment with initial colonizers followed by cell-to-cell adhesion with secondary colonizers occurs on the implant surface [276].

Biofilms are the preferred method of growth for most bacteria because they facilitate exchange of nutrients and protect the bacterial community from competing microorganisms [277].

Whilst biofilms are often associated with negative implications, it should be noted that they occur naturally in our environment and it is only when the balance of virulence factors imbalances others that pathology results.

Similar to the healthy periodontium around natural teeth, the microorganisms associated with healthy implants are predominantly Gram-positive cocci and rods microorganisms [274] [278] [279]. The dominant species are members of the yellow and purple complexes or are independent of the complexes such as *Actinomyces naeslundii or Actinomyces viscosus* [280] [281] . Gram-negative bacteria can be found in smaller proportions and include *Prevotella intermedia, Porphyromonas gingivalis, Tannerella forsythia, Prevotella nigrescens*, and *Campylobacter rectus* [280].

This suggests that certain species are indigenous, host-compatible organisms. As demonstrated in an experimentally induced peri-implant mucositis study in humans, plaque accumulation and development of peri-implant mucositis were comparable at implant and natural teeth sites [282]. Interestingly, with a similar amount of plaque accumulation, implant sites had increased host response and proinflammatory cytokine production compared with that found in teeth, suggesting that implants may act as a foreign body [283].

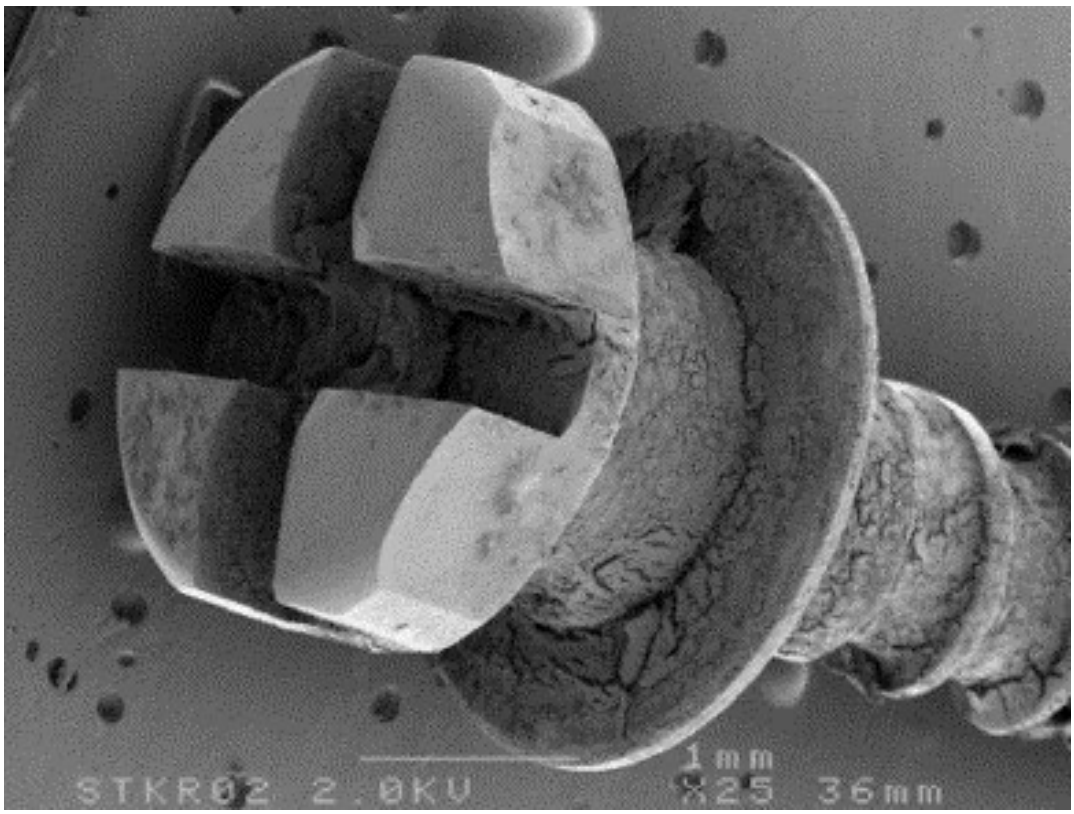


Figure 1.10 : Scanning electron micrograph of a clinically failed OMSI (Ti6Al4V). There is a thick biofilm present covering all aspects of the OMSI.

Source : Chin[284]

1.7 Surface roughness and the effect of biofilm formation

There has been much work completed regarding the relationship between successful osseointegration dental implants and increased surface roughness. The literature informs us that indeed, increased surface roughness is correlated with increased osseointegration [285] [286] [287].

However, it has also been suggested that, a higher surface roughness (Ra value > 0.2) increases biofilm formation [288] [289]. This may lead to the accelerated progression of peri implant disease [290] [291] [292].

In their experimental study with dogs, Berglundh et al. [292] revealed that rougher surfaces when compared with polished machined surface implants resulted in increased plaque formation and faster progression of peri-implantitis.

These finding were echoed by Amarante et al. [293] who illustrated that machined surface implants harboured significantly less bacteria than plasma-sprayed implants.

The abutment surface roughness of dental implants is also thought to affect the aggregation of biofilm. With increasing abutment surface roughness, higher supra mucosal plaque accumulation has been noted [294].

Quirynen et al. [295] in their examination of 9 patients found that abutments with a rough surface harboured up to 25 times more microorganisms than was found in the supra mucosal plaque of rough abutments compared with smooth abutments [295].

Much of the available literature pertains to peri implantitis and the infection of conventional dental implants. There is limited clinical information regarding the infection of OMSIs in the current literature.

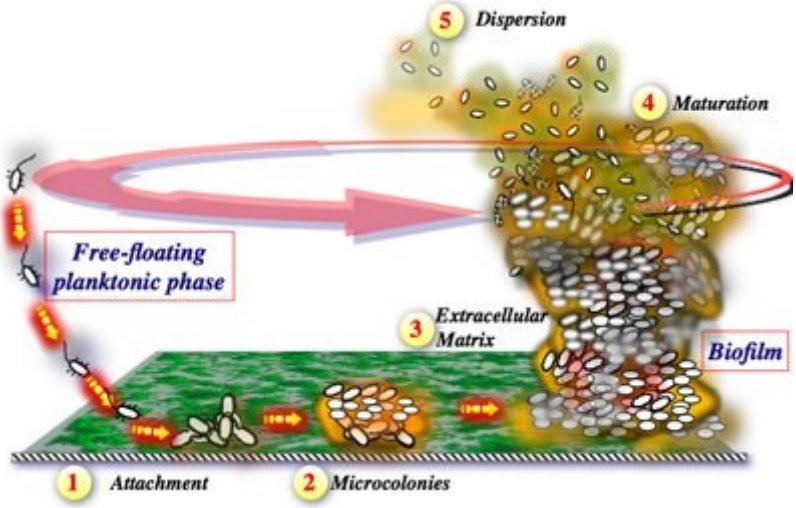


Figure 1.11 : Diagrammatic representation of biofilm formation. 1 - Attachment, 2 –Micro colonisation. 3 – The development of an extracellular matrix. 4, 5 – Maturation and dispersion of the biofilm.

Source : Chin[284]

1.8 Antimicrobial agents on orthodontic biofilms

Orthodontic appliances reduce the ability of patients to maintain oral hygiene effectively. The introduction of OMSI is no different with the removal of biofilm from *in situ* OMSI made difficult. Whilst aggressive mechanical debridement may jeopardize the retention of the OMSI, many clinicians suggest the use anti-microbial containing mouth rinses to prevent postoperative peri-implantitis [9] [200].

These agents generally have 4 broad mechanisms of disrupting dental biofilm formation [296];

(1) Prevention of the adherence and build-up of a biofilm.

(2) Destruction of an existing biofilm.

(3) Reducing the growth processes in the biofilm.

(4) Destruction of individual microorganisms in the biofilm.

Chlorhexidine is regarded as the most representative of the chemoprophylactic agents, and is widely considered to be the most effective agent against plaque and gingivitis [297] [298]. It is extensively studied, and one of the most frequently utilised bactericides in dentistry [299] [298].

Chlorhexidine is a bisbiguanide antiseptic and acts by binding to the bacterial cell membrane and causing rupture by interference to osmosis [300]. As well as this, Chlorhexidine has shown some ability to help inhibit adherence of microorganisms to a surface thereby preventing growth and development of biofilms [301]. Chlorhexidine has also been shown to prove effective against fungi, bacterial spores, and protozoa [300]. The excellent substantively of chlorhexidine is one of its main advantages in providing continuous antimicrobial action [302].

Contrastingly, fluoride is universally accepted as a highly effective anti-caries agent. This anti-caries action is delivered directly to the dental hard tissues through the process of acid mediated demineralization and remineralization [303] [304]. However, it is also accepted that fluoride has a direct bacteriostatic effect thereby leading a two pronged approach [303]. The literature reflects that fluoride may affect bacterial metabolism through a set of actions with fundamentally different mechanisms. It can act directly as an enzyme inhibitor, for example for the glycolytic enzyme enolase, which is inhibited in a quasi-irreversible manner [302] [305].

Direct action seems also to occur in inhibition of heme-based peroxidases with binding of fluoride to heme. The flavin-based peroxidases of many oral bacteria are insensitive to fluoride.

Furthermore, another mode of action involves formation of metal–fluoride complexes. Moreover, several metal-fluoride complexes can be formed on titanium implant surfaces such as CuF_2 , SnF_2 , Al_2F_3, and TiF_4. These complexes are responsible for fluoride inhibition of proton-translocating F-ATPases and are thought to act by mimicking phosphate to form complexes with ADP at reaction centres of the enzymes [306]. This exhibits direct antimicrobial effects against oral bacteria.

Chapter 2

Significance and aims of study

2.1 Significance

Whilst many factors have been implicated in the grossly variable stability of OMSI, biological interaction between the metal body and organic bone is pivotal. Various processes exist for the manufacture of OMSI with surface treatment of the implant varying from manufacturer to manufacturer.

Anodic oxidized implants are one such group which have perceived greater surface roughness than machined un-treated implant surfaces [307]. Because osteoblasts more easily attach to a rough surface than a smooth surface [308] [309], this may make such a surface coating advantageous for OMSI. However, treating the entire screw with such a coating also makes the head and neck of the screw equally conducive to biofilm aggregation – a factor shown to affect the stability and success of OMSI [9].

In addition, the phenomenon of peri implantitis is well documented in the literature with respect to conventional dental implants [310] [311] [263]. In the case of restorative dental implants, care is taken to ensure that any attempt to roughen the surface of the implant does not extend onto the trans - mucosal collar as this will accelerate peri implantitis.

Contrastingly, OMSI are available in a myriad of materials and surface treatments. Anodic oxidized titanium alloy OMSIs are one example of various materials and finishes available in the current market. Others include stainless steel, machined titanium alloy, and commercially pure titanium.

Whilst there has been much work performed on surface roughness of conventional dental implants, and it's correlation with bone healing [312] to date, the investigation into the various surface characteristics of various OMSI systems has been limited. To the author's knowledge, there has been limited recent work conducted exploring the surface roughness of commercially available OMSI systems and its effect on biofilm formation [284 313].

2.2 Aims

This study aimed to compare the surface roughness of four commercially available orthodontic miniscrew implants and their effect on biofilm formation in an *in vitro* setting. The null hypotheses state that:

- There will be no significant difference in surface morphology among implant groups.

- There will be no significant difference in the growth of biofilm among implant groups.

- There will be no significant difference in the killing efficacy of antimicrobial agents.

Chapter 3

Materials and method

3.1 Materials

Four commercially available, self-tapping orthodontic mini implants that are commonly available in the Australian market were selected for the present study. The details are provided in the table below.

Table 3.1 : Summary of implant groups used for this experiment.

Product	Material	Surface	Length (mm)	Diameter (mm)	Production lot	Manufacturer
Aarhus	Ti6Al4V	Anodised	9.2	1.5	095/8/20011	American Orthodontics
Vector TAS	Ti6Al4V	Anodised	10.0	2.0	072213	Ormco
Leone	Surgical stainless steel (ISO 5832/1)	Machined	10.0	2.0	000-1510-02	Leone
TOMAS®	Ti6Al4V	Machined	10.0	2.0	431873	Dentaurum

All micro-implants were individually packaged and sterilized by the manufacturers. Thus by opening the packages only at the start of each experiment, and using careful handling, contamination of the OMSI surface was prevented. The exception was the Leone stainless steel implants which required sterilization prior to use. They were handled in a similar manner as described after sterilization in a Class B Steam Autoclave.

3.2 Surface roughness

The surface roughness of each group of OMSI was measured by atomic force microscopy (AFM). AFM utilizes a high-resolution, scanning microscope capable of imaging and measuring samples on the nanometer to angstrom scale. A fine probe on a cantilever is used to scan the surface of a specimen. Forces generated as the tip of the probe interacts with the surface are recorded as deflections of the cantilever.

Four implants were selected from each group, with each implant subject to analysis using an INTEGRA Modular AFM (NT-MDT, Moscow, Russia) available at the Advanced Analytical Centre of James Cook University, Townsville.

Measurements were taken at the head and neck of the implant, and repeated 4 times on each OMSI. Data was output both in graphical and numerical format with *Sa* representing the average distance of the roughness profile to the centre plane of the profile. There were 16 measurements per implant group.

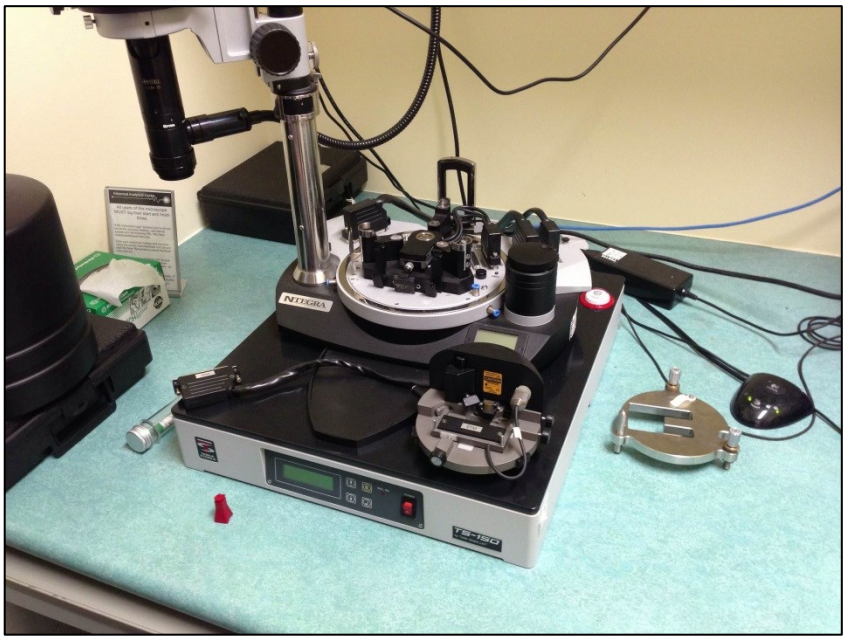


Figure 3.1 : The INTEGRA Modular Atomic Force Microscopy Unit (NT-MDT, Moscow, Russia). A unit was available in the James Cook University Advanced Analytical Centre, Townsville

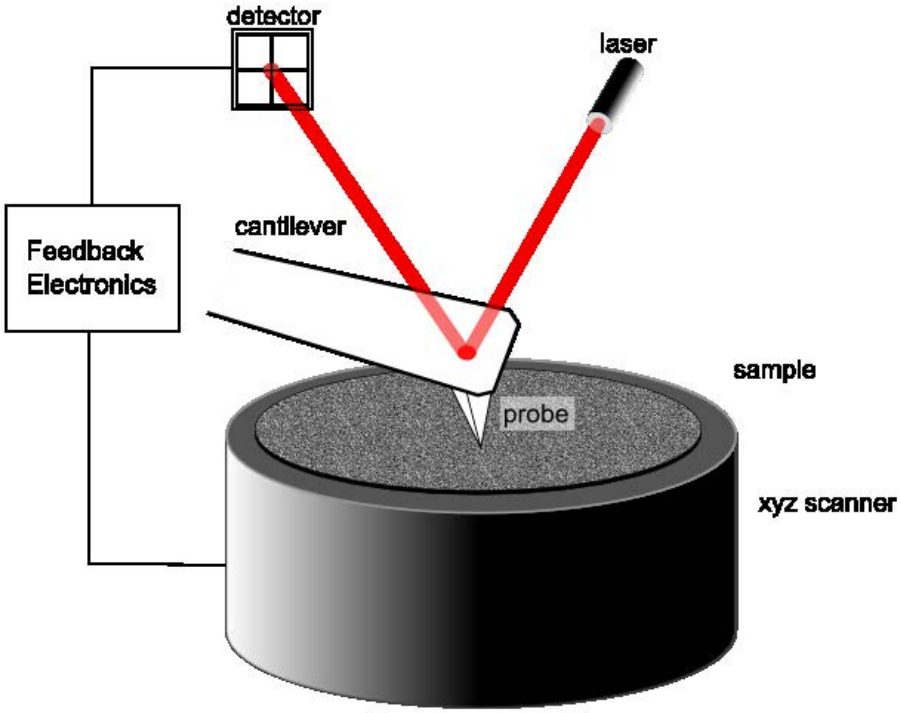


Figure 3.2 : Schematic diagram of how an AFM works.

Atomic force microscopy (AFM) utilizes a high-resolution, scanning microscope capable of imaging and measuring samples on the nanometer to angstrom scale. A fine probe on a cantilever is used to scan the surface of a specimen. Forces generated as the tip of the probe interacts with the surface are recorded as deflections on the cantilever.

Source: Adapted from www.wikipedia.org

3.3 Surface morphology

In addition, surface characteristics of each OMSI group were investigated qualitatively using scanning electron microscopy (SEM).

One OMSI from each group was selected to be imaged. There were a total of four OMSI used for this experiment, one from each group. Each OMSI was fixed onto a SEM stub holder and sputter coated prior to analysis. Images at set magnifications were taken by using a Jeol JSM5410LV (Peabody, Massachusetts, USA) available at the Advanced Analytical Center of James Cook University.

This was a tungsten gun SEM with secondary electron imaging (SEI), backscatter electron imaging (BEI) and a cathodoluminescence detector (CL). Images were compared for qualitative analysis.

3.4 Biofilm growth on implant surfaces

To grow biofilms on OMSIs, human saliva was collected from 10 male and 10 female volunteers. Saliva secretion was stimulated by chewing orthodontic elastics (Ormco). They were collectively pooled and stored in ice chilled test tubes (50mL). All saliva collection was completed within 24 hours of the experiments being conducted with a total of 20 donated samples.

To prevent protein break down, phenylmethylsulfonylfluoride (PMSF) (Sigma Aldrich) was added to collected saliva to make a final concentration of 1.0 mM.

Saliva collection was conducted with the approval of the ethics committee of James Cook University (Approval number: H5309, Refer Appendix A). All volunteers were issued an information sheet regarding the saliva collection procedure (Appendix B), prior to signing a consent form (Appendix C).

One OMSI from each group were selected and prepared as discussed by sterilization in a Lisa 500 (WH, Bürmoos, Austria) at 134°C (206 kPa) for 45 minutes. Each OMSI was immersed in 1.0 mL of freshly pooled saliva in individual Eppendorf tubes.

The biofilm was grown by culturing the collective samples in an aerobic incubator M-20AIC (Sanyo, Loughborough , UK). Incubation occurred at 37° C for 24 hours in line with parameters suggested by previous studies [284 314 315].

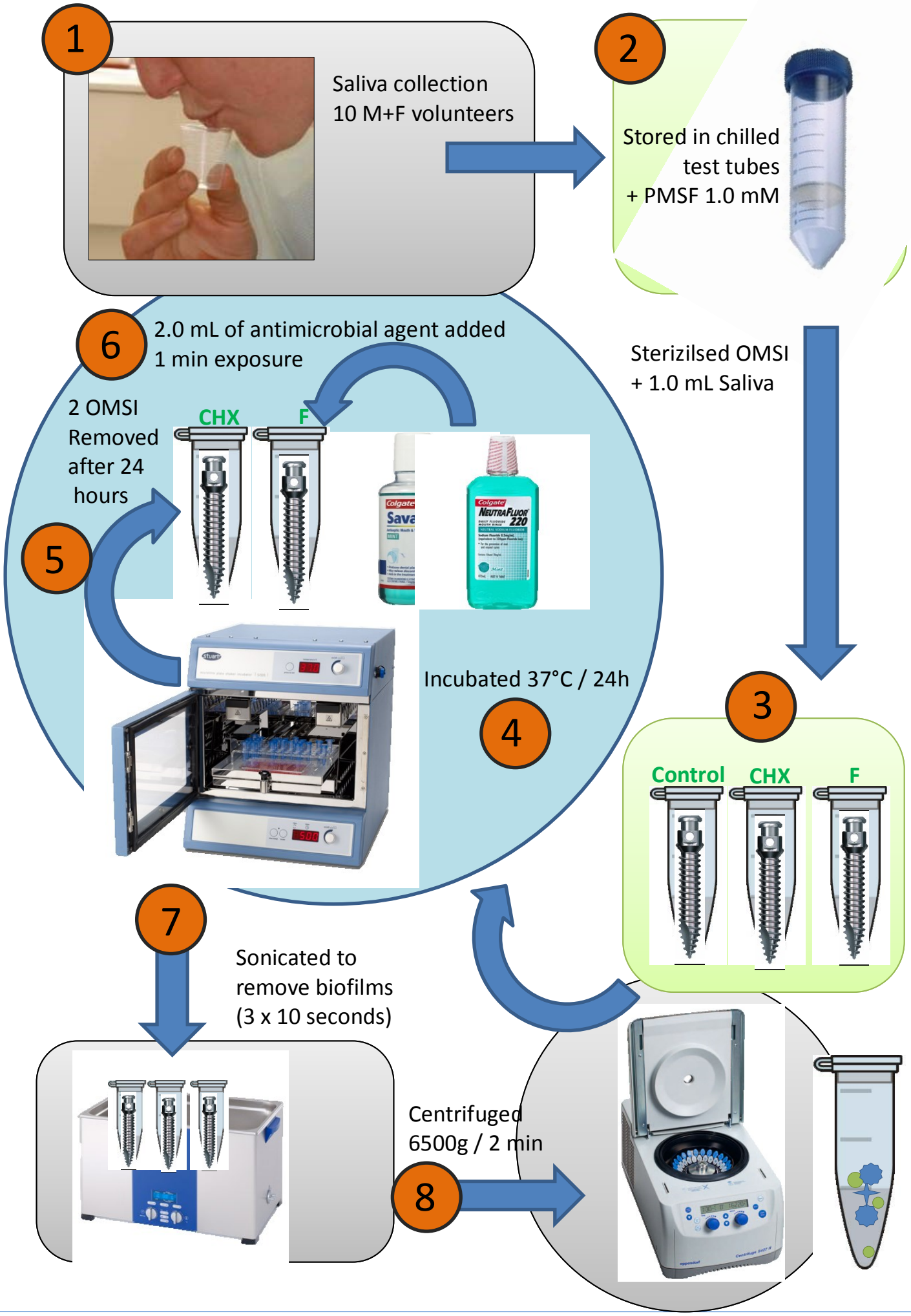
In addition, it was intended to simulate the maximum period of time biofilms may be allowed to grow on such a surface.

After incubation, in order to remove the bacteria off the surfaces, the OMSI with adhering biofilms were merged in 1.0 mL of sterile adhesion buffer (50mM potassium chloride, 2 mM potassium phosphate, 1mM calcium chloride, pH 6.8) and sonicated (Bandeiln SONOREX Super, Sigma Aldrich) for approximately 3 x 10s, with 30s intervals in an ice water bath.

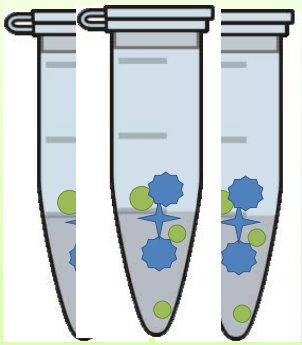
The bacterial pellet was collected after a subsequent centrifuge at 6500g for 2 min at 10°C, which was re-suspended into 50 µL of sterile adhesion buffer.

Furthermore, to test the antimicrobial efficacy of 0.2% chlorhexidine (Colgate, AUSTRALIA) and 0.055% fluoride rinses (250 ppm F, Colgate, AUSTRALIA), two samples of each micro-implant were prepared for this experiment. After 24 hours of incubation, the each implant was immersed in 2 mL either mouth rinse for 1 min, respectively.

Figure 3.3 : Experimental procedure to culture biofilms on the implant surfaces.



9



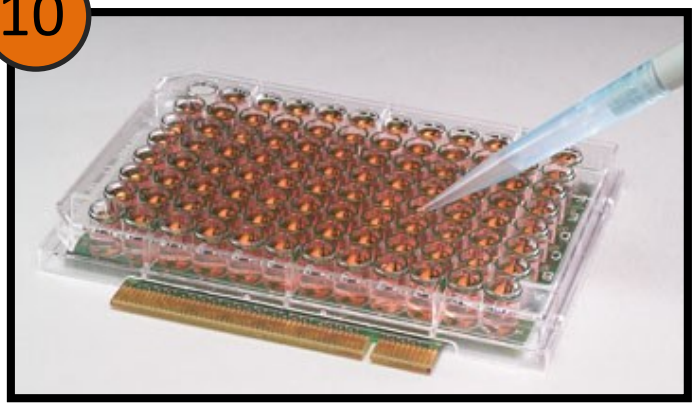
Re suspended
Bacteria containing
samples

200 ul of sample

11

Chemical Staining
2uL of Thiazole Orange
2uL of Propodium Iodide
20uL of counting beads

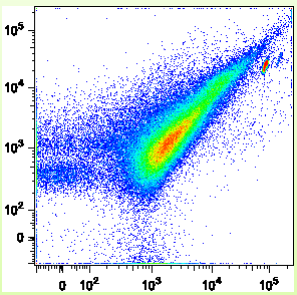
10



14 Repeat experiments
7 times

12 Processed in
BD FACS

13



Output data

The bacteria from all incubated implants were then quantified using flow cytometry (FCS). Flow cytometry is a laser-based, biophysical technology employed for the purposes of cell counting, cell sorting, biomarker detection and protein engineering. It works by suspending cells in a stream of fluid and passing them by an electronic detection apparatus.

It allows simultaneous multi-parametric analysis of the physical and chemical characteristics of up to thousands of particles per second. This makes flow cytometry both efficient and accurate.

Prior to analysis, 200 µL of bacterial suspension was mixed with 20 µL of immunofluorescent counting beads and 4 µL of a staining medium from the BD Cell Viability Kit in a multi well plate. This included 2µL of Thiazole orange TO (42 µmol/L in dimethyl sulfoxide (DMSO) and 2µL of Propidium iodide PI (4.3 mmol/L in water).

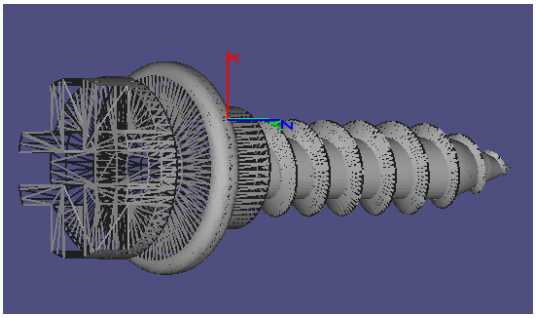
This mixture in the multi well plate was analyzed by a BD FACSCanto II (San Jose, CA, USA) available in the James Cook University Analytical Flow Cytometry Unit. Data was exported to FlowJo LLC Data software (Oregon, USA) for analysis.

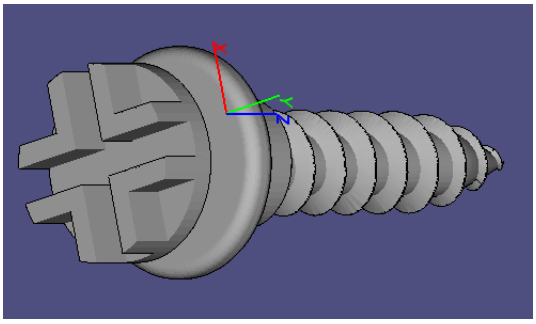
The experiments were repeated seven times with separately pooled saliva as outlined in the collection protocol and new micro-implants.

To calculate the amount of bacteria grown per mm^2 on each of the implant groups, the area of each OMSI was measured. Where possible, 3D scanning of the screws along with manufacturers data was used to obtain accurate measurements of the total surface area of each OMSI. This allowed direct comparison between the different groups of OMSI. To capture the 3D models, a DOF white light scanner was used (Seoul, Korea) together with PowerShape (Cambridge, England) and Materialise (Leuven, Belgium) 3D software.

One-way ANOVA was completed to compare the means of total bacterial formation on each implant group. Post-hoc analysis included Tukey's correlation.

Furthermore, paired t-tests were conducted between the chlorhexidine and fluoride groups to determine the mean amount of dead bacterial proportion. This provided an insight into killing effectiveness of each of the agents.





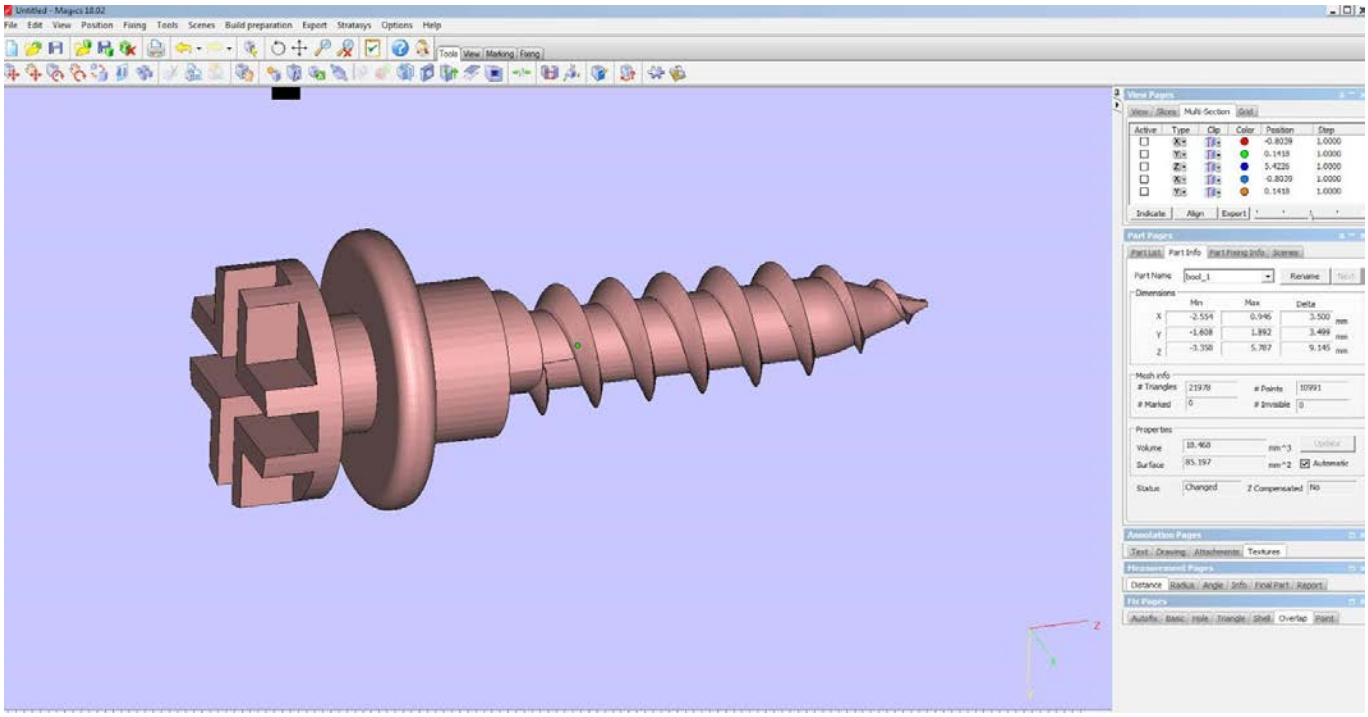


Figure 3.4 : 3D models generated from scanning of the Aarhus OMSI Total surface area was thus calculated more accurately.

3.5 Statistical analysis

A Shapiro Wilk test was used prior to analysis of variance of means (ANOVA) to evaluate the data sets for normality. Values of skewness and kurtosis were calculated to help identify outlying values which could be removed to ensure similar distributions were being compared.

Multiple analyses were conducted using one-way ANOVA, followed by Tukey's *post hoc* test.

A Student's t-test was used to calculate the difference between Chlorhexidine and Fluoride killing efficacy across groups.

Prism 6 (GraphPad Software, La Jolla, CA USA) was used as the statistical program to run these analyses. $p < 0.05$ was considered significant for all test utilized.

Chapter 4

Results

4.1 Summary of OMSI groups used

Table 4.1 : Summary of the groups of OMSI used

ID	Product	Material	Surface	Length (mm)	Diameter (mm)	Area (mm^2)	Production lot	Manufacturer
1	**Aarhus**	Ti6Al4V	Anodised	9.2	1.5	85.2	095/8/20011	American Orthodontics
2	**Vector**	Ti6Al4V	Anodised	10.0	2.0	62.5	072213	Ormco
3	**Leone**	Stainless steel (ISO 5832/1)	Machined	10.0	2.0	72.0	000-1510-02	Leone
4	**TOMAS®**	Ti6Al4V	Machined	10.0	2.0	88.16	431873	Dentaurum

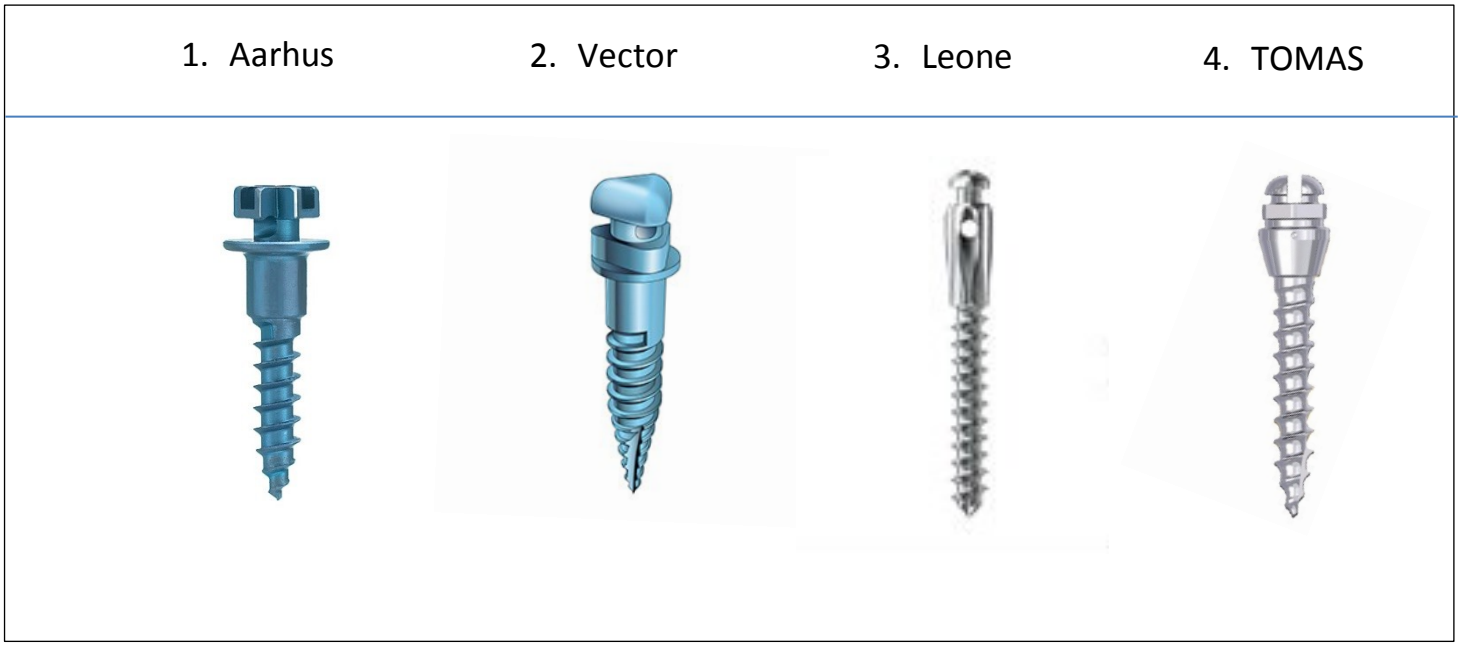


Figure 4.1 : Examples of the OMSI used for the experiment

4.2 SEM qualitative analysis

Morphological analysis under SEM showed that four of the implant groups exhibited varying surface finishes. This reflected their varying composition.

The TOMAS group which was manufactured from machined titanium alloy exhibited imperfections on the surface. There was evidence of divots, and pitting. In addition what appeared to be striations were evident where the metal had been cut.

Leone group which was manufactured from stainless steel also resembled these imperfections. The pitting appeared to be more severe, especially where the smooth metal transitioned into the thread. These imperfections on the OMSI surfaces were attributed to the manufacturing process.

The Vector group which was manufactured from titanium alloy and finished with anodic oxidation exhibited unique surface morphology in that its surface when imaged at the head and neck appeared roughest. There were numerous striations also concluded to be originating from the manufacturing process. This roughened pattern appeared to be consistent throughout the length of the Vector screws with a much denser configuration.

Interestingly, the Aarhus group which is also manufactured from titanium alloy, and finished with surface adonization did not exhibit such characteristics. It appeared smoothest with less gouging of the metal where cut lines appeared. Furthermore, the presence of pitting was virtually absent in the anodized titanium alloy samples analysed.

This qualitative analysis indicates that with respect to the measured OMSI groups, the Vector brand possess the roughest surface characteristics.

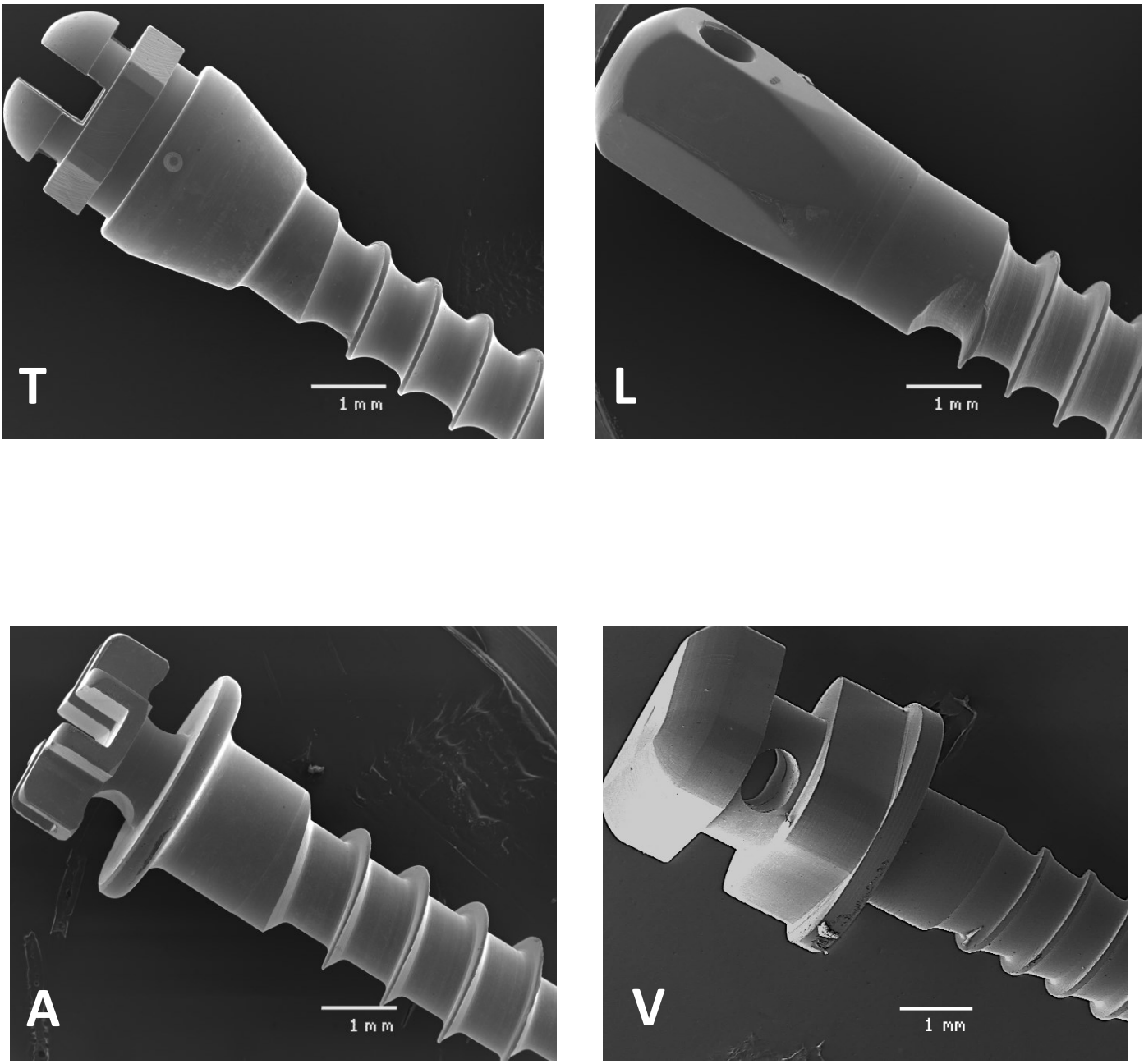


Figure 4.2 : Example of SEM images at various magnifications.
A: Aarhus, L: Leone, V: Vector, T: TOMAS

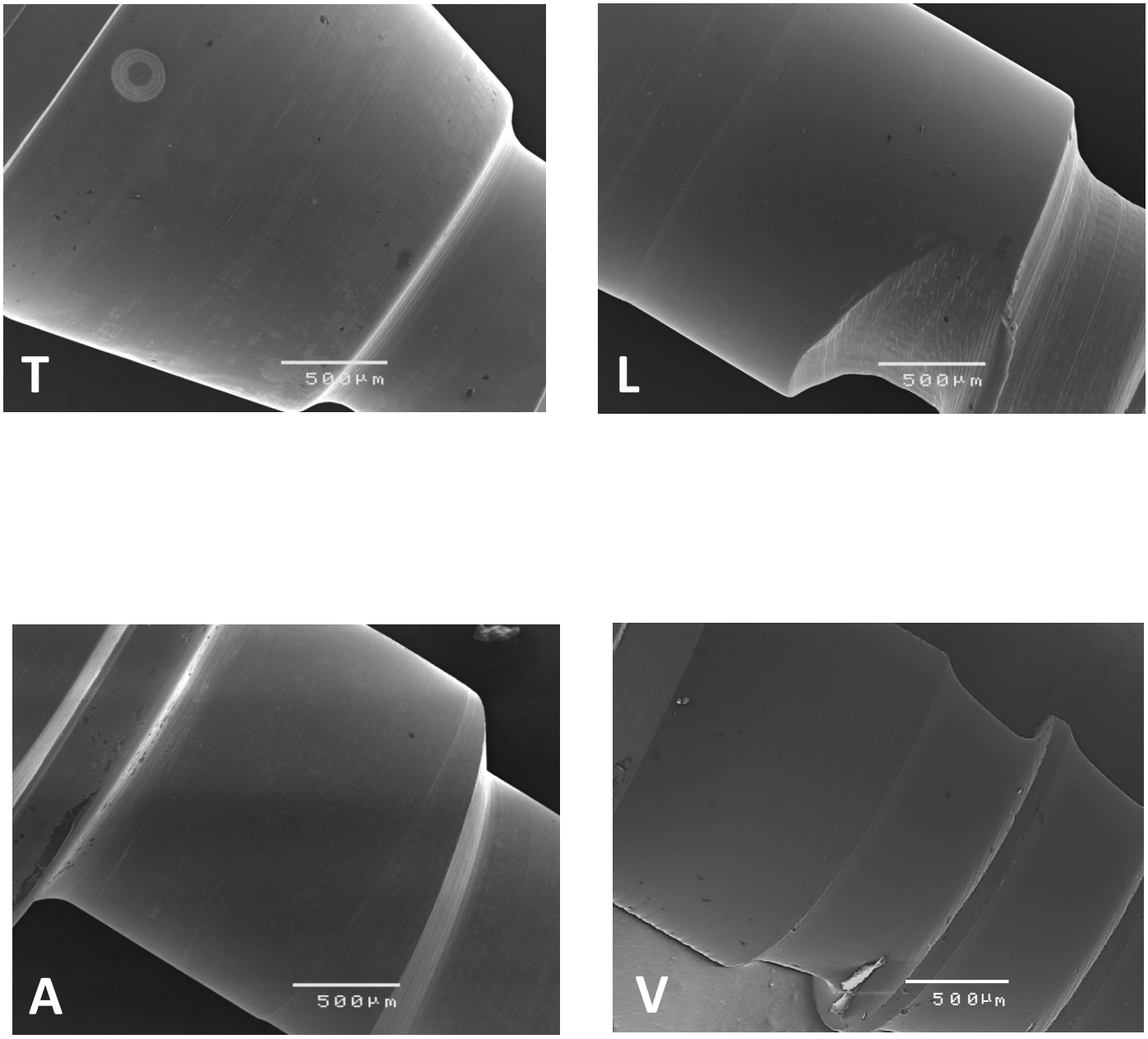


Figure 4.3 : Example of SEM images at various magnifications.
A: Aarhus, L: Leone, V: Vector, T: TOMAS

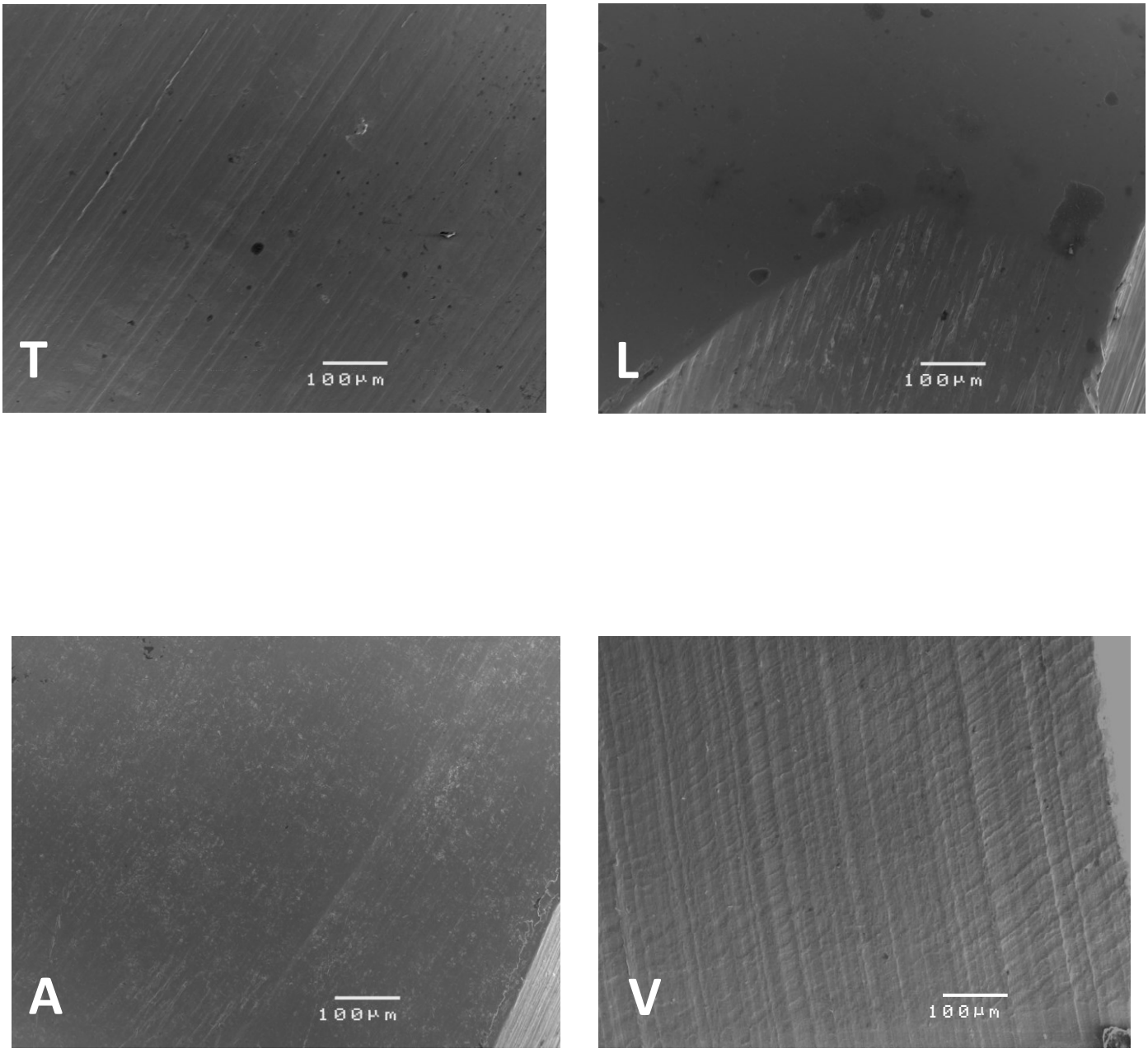
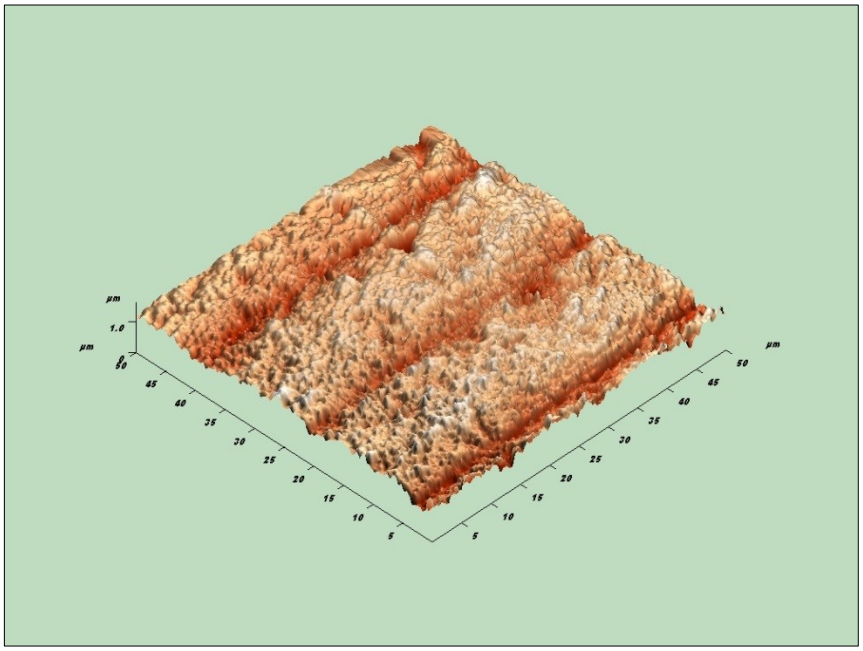


Figure 4.4 : Example of SEM images at various magnifications.
A: Aarhus, L: Leone, V: Vector, T: TOMAS

4.3 Surface roughness

The AFM outputs visual as well as numeric data. Visual data reflects the surface topography of a section of the sample while the numerical data presents an average roughness. Complete raw data in appendix C.



Sample : A1-001

Amount of sampling	65536	Indicates number of sites measured
Max	1661.25 nm	Maximum roughness measured
Min	0 nm	Minimum roughness measured
Average Roughness, Sa	***128.38 nm***	Average roughness was used

Figure 4.5 : Example of visual and numerical output for typical AFM sample.

Aarhus surface roughness plots

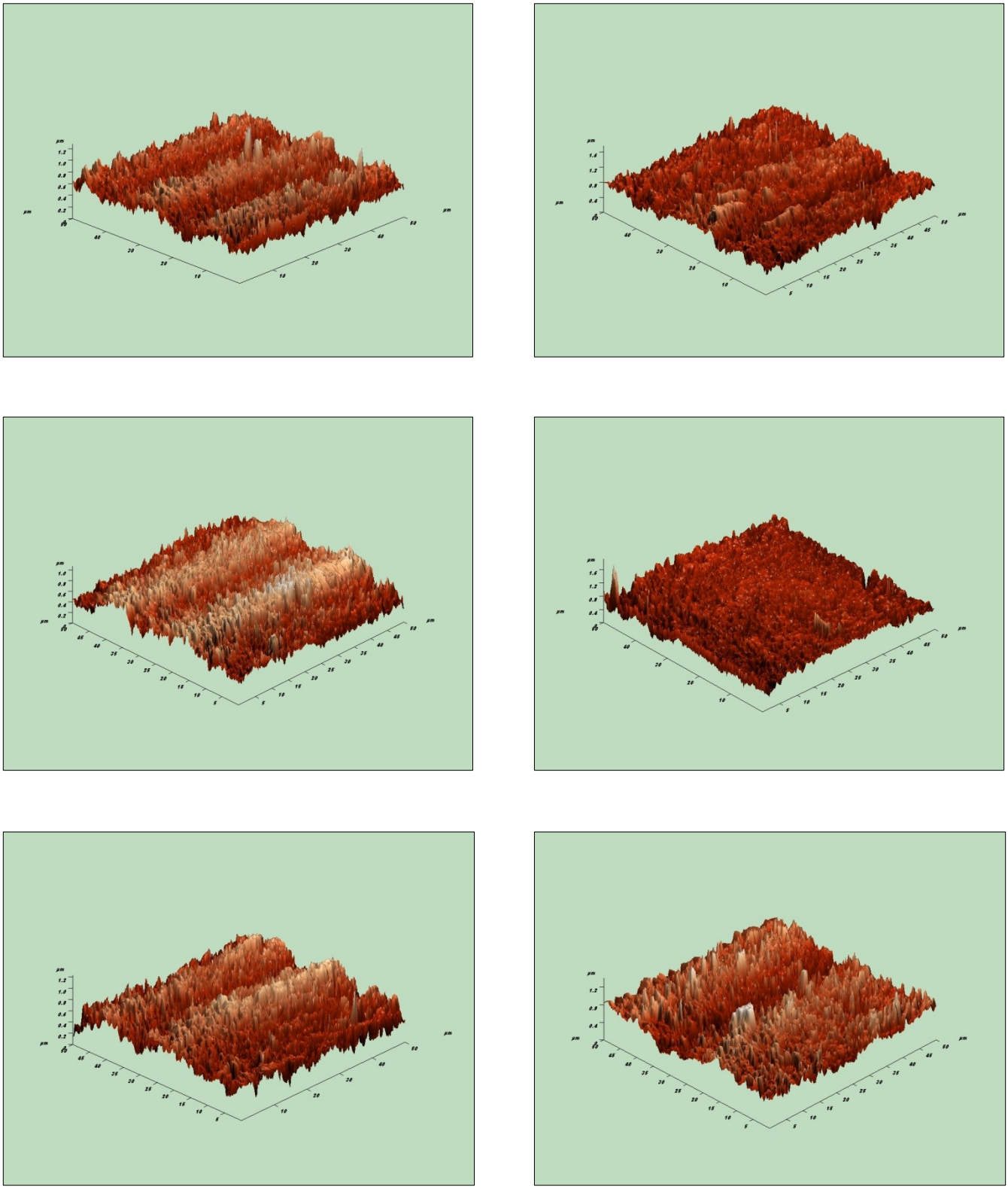


Figure 4.6 : Representation of surface roughness as measured by AFM for **Aarhus**

Vector surface roughness plots

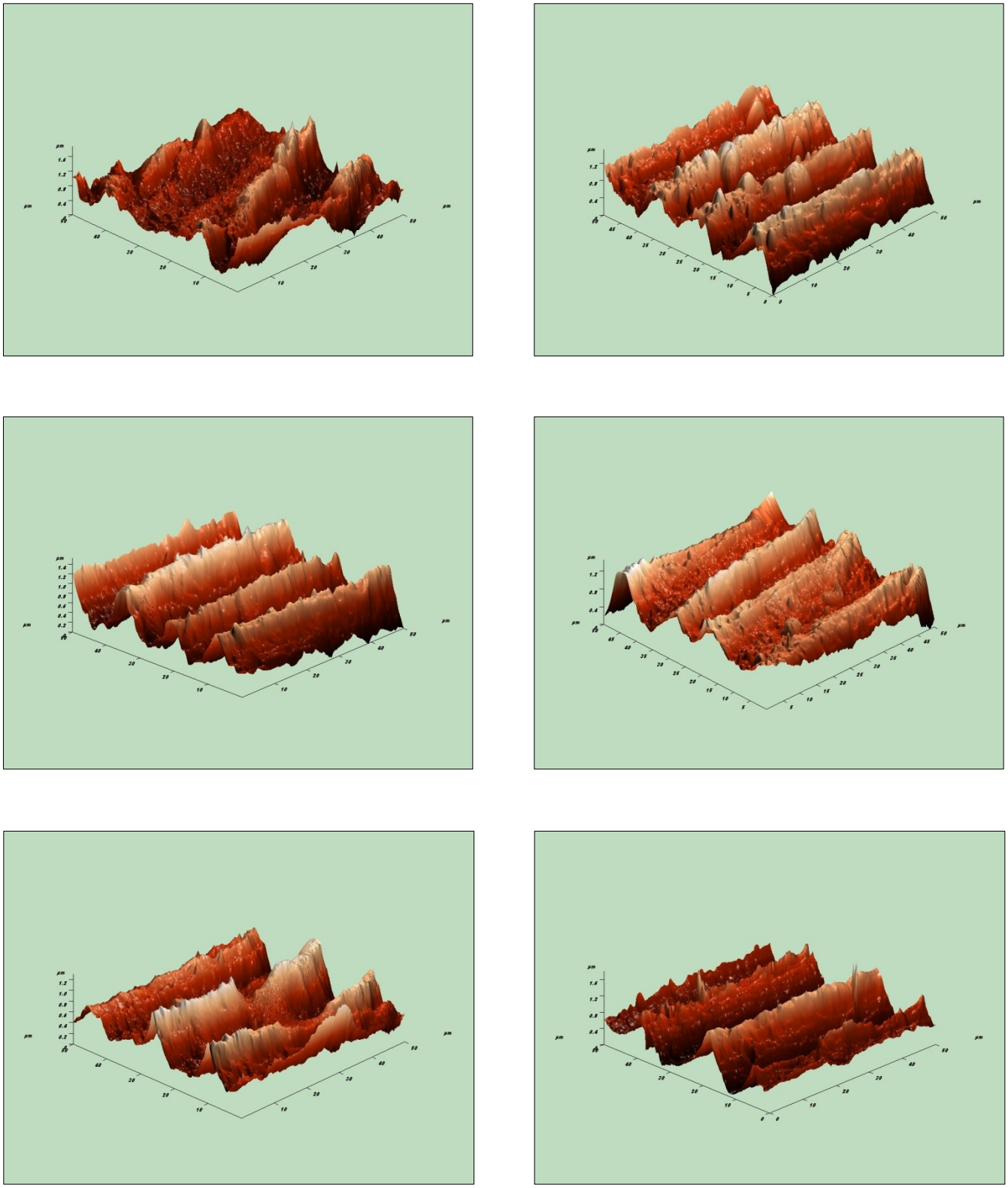


Figure 4.7 : Representation of surface roughness as measured by AFM for **Vector**

Leone surface roughness plots

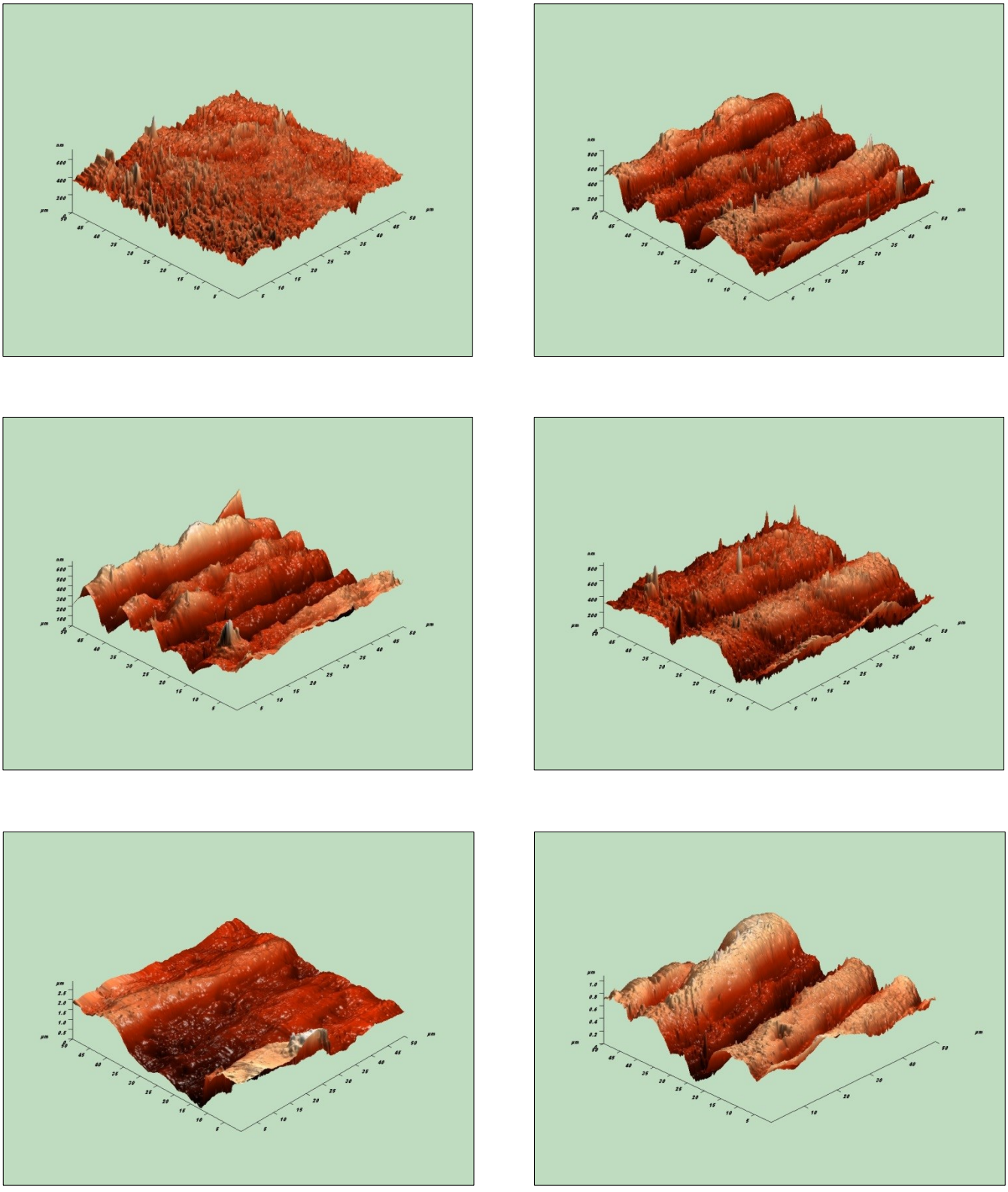


Figure 4.8 : Representation of surface roughness as measured by AFM for **Leone**

TOMAS surface roughness plots

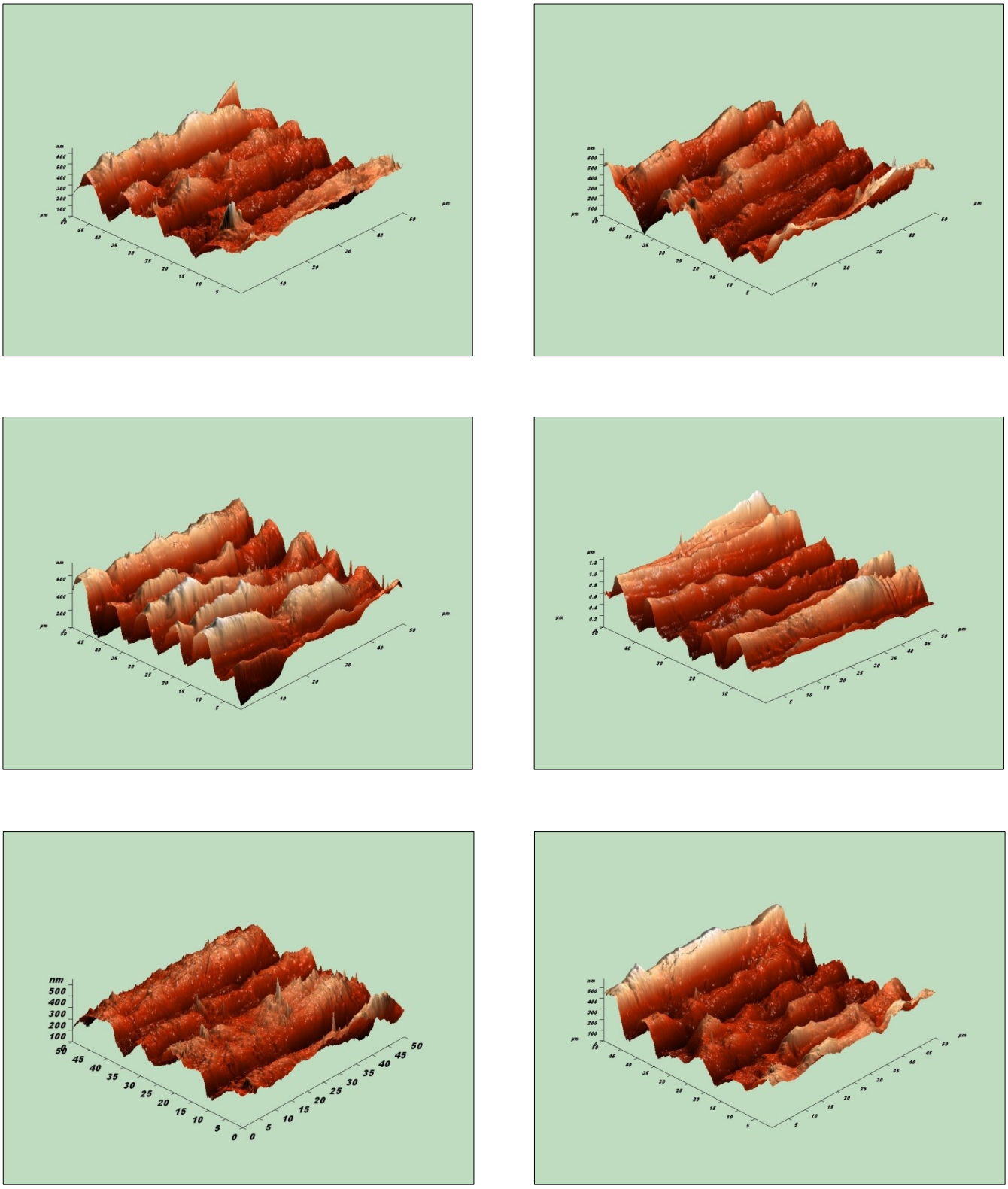


Figure 4.9 : Representation of surface roughness as measured by AFM for **TOMAS**

4.4 Surface roughness – Statistical Analysis

ANOVA (Analysis of variance of means) was chosen to compare the mean roughness between the four implant groups. Prism 6 (GraphPad Software, La Jolla, CA USA) was used as the statistical program to run these analyses.

Table 4.2 : ANOVA: Descriptives of investigated implant groups

Descriptives

	Vector	Aarhus	TOMAS	LEONE
Number of values	16	16	16	16
Mean roughness	239.4 ± 72.10*	127.8 ± 30.24	105.9 ± 72.10	119.9 ± 49.02

* indicates $p<0.05$

Table 4.3 : ANOVA: Roughness of investigated implant groups

Roughness

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	181239.14	3	60413.04	15.62	**.00**
Within Groups	232013.77	60	3866.89		
Total	413252.91	63			

Table 4.4 : ANOVA Multiple comparisons showing Roughness of investigated implant groups

Dependent Variable: Roughness (nm)
Statistical test : One way ANOVA with post-hoc Tuckey analysis

Group	Group	Mean Difference	Std. Error	Sig. (P)	95% Confidence Interval	
					Lower Bound	Upper Bound
Aarhus						
	Vector	-111.63*	21.99	**.00**	-171.62	-51.64
	TOMAS	21.92	21.99	1.00	-38.07	81.91
	Leone	7.84	21.99	1.00	-52.15	67.83
Vector						
	Aarhus	111.63*	21.99	**.00**	51.64	171.62
	TOMAS	133.55*	21.99	**.00**	73.56	193.54
	Leone	119.47*	21.99	**.00**	59.48	179.46
TOMAS						
	Aarhus	-21.92	21.99	1.00	-81.91	38.07
	Vector	-133.55*	21.99	**.00**	-193.54	-73.56
	Leone	-14.08	21.99	1.00	-74.07	45.91
Leone						
	Aarhus	-7.84	21.99	1.00	-67.83	52.15
	Vector	-119.47*	21.99	**.00**	-179.46	-59.48
	TOMAS	14.08	21.99	1.00	-45.91	74.07

* indicates $p<0.05$.
$F_{(3, 60)} = 15.63$

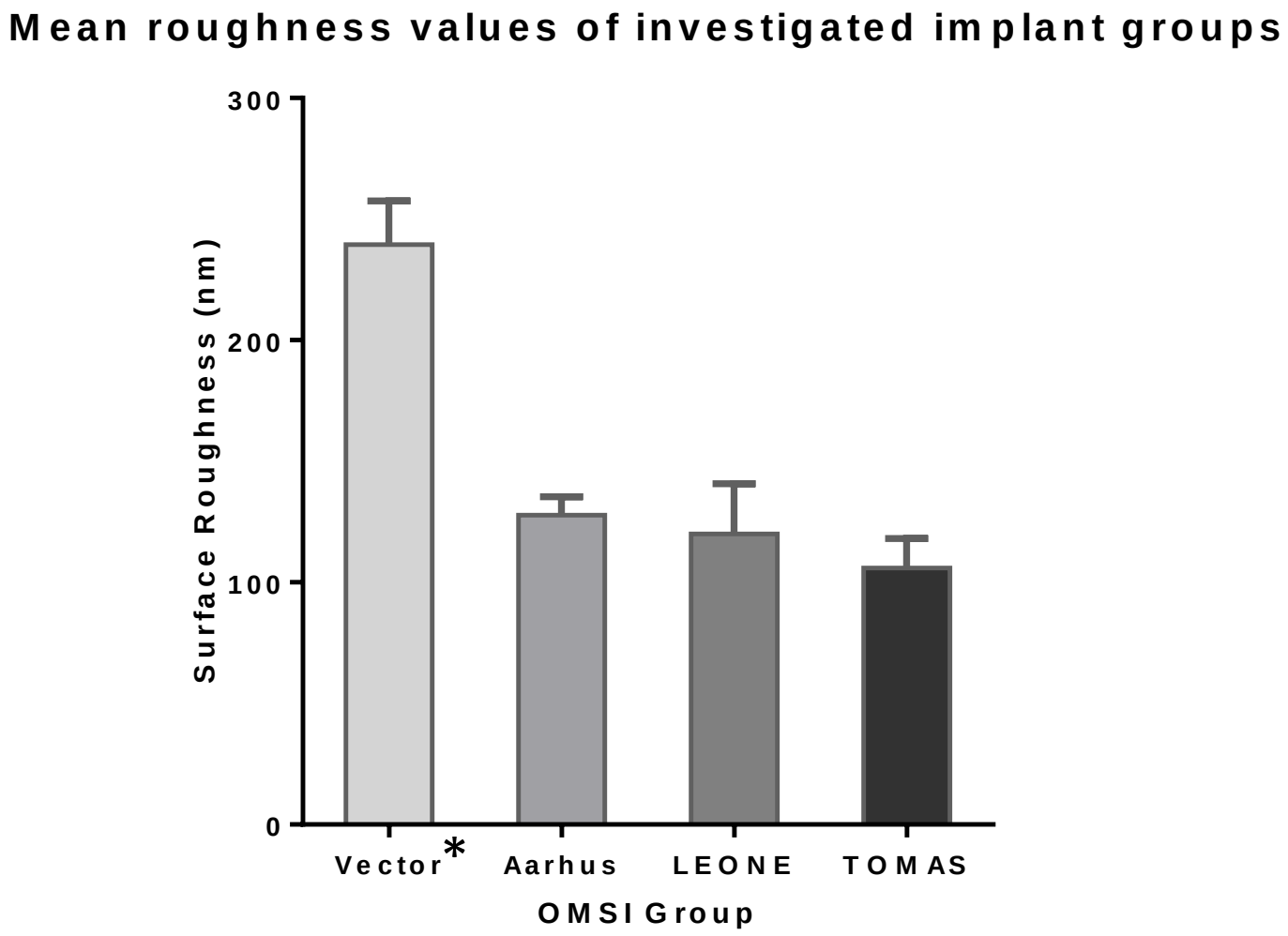


Figure 4.10 : Box plot showing the mean roughness of investigated implant groups. * indicates $p<0.05$

4.5 Bacterial growth on implant groups

Data was visually output from the BD FACSCanto II BD FACSCanto II (San Jose, CA, USA) as graphical data. The image in Figure 4.11 illustrates a typical data set whereby the scatter is reported on the y axis, and the bacteria on the x axis. Using FCS, the scatter of a particular wavelength can help to determine a particular stained substance. In this case, live and dead bacteria.

The total bacteria are represented by the collective density in the centre of the image. The immunofluorescent beads can be seen just on the right hand side grouped together as a cluster. Similarly, data was represented in histogram format whereby the amount of bacteria could be quantified once calibrated with the known quantity of immunofluorescent beads.

Figure 4.12 illustrates a sample graph where no intervention was used. 98% of the cell population are alive with only a small number of reported dead cells. Propidium Iodide binds to the dead bacteria and hence describes their numbers. This shows up on the histogram as a second spike in the wavelength on the right hand side. It is evident that there are a low proportion of dead bacteria. However, Figure 4.13 shows a higher proportion of dead bacteria due to an intervention of chlorhexidine as described earlier.

Finally Figure 4.14 shows a similar pattern but slightly lesser so. This data was manipulated in FlowJo LLC Data analysis software (Oregon, USA). Numerical figures of bacterial numbers were ascertained, and further statistical analysis carried out in Prism 6 (GraphPad Software, La Jolla, CA USA).

The mean bacteria per mm^2 of each implant group was carried out by using the number of bacteria divided by the total surface area of the respective implant group. The total bacteria were represented by the sum of live and dead bacteria.

Graph showing scatter Vs. thiazole orange stain (Sample B1)

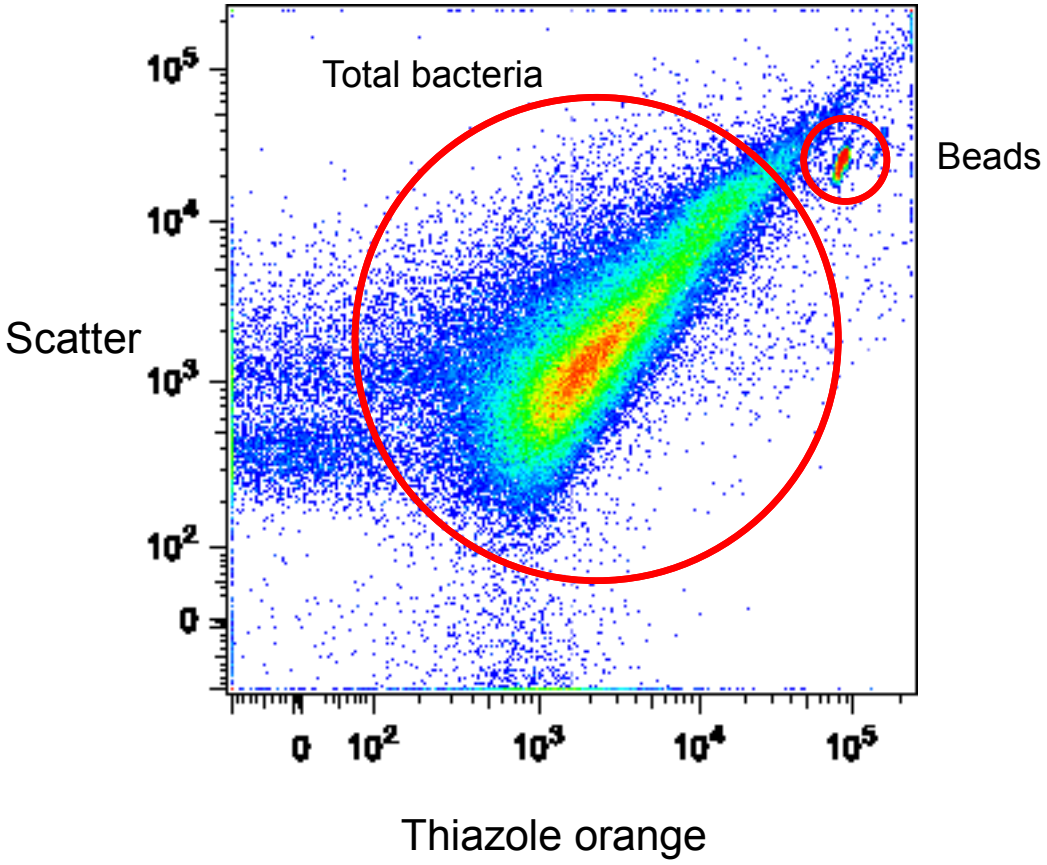


Figure 4.11 : Graph showing scatter vs. thiazole orange stain (Sample B1)

Red indicated dense bacterial concentration the spectrum reduces as we move from red towards the periphery indicating fewer bacteria. The wavelength of the immunofluoresent beads is known and they show up on the right (circled) as a constant.

Graph showing total number of cells vs. propidium iodide (Sample B1 – No intervention)

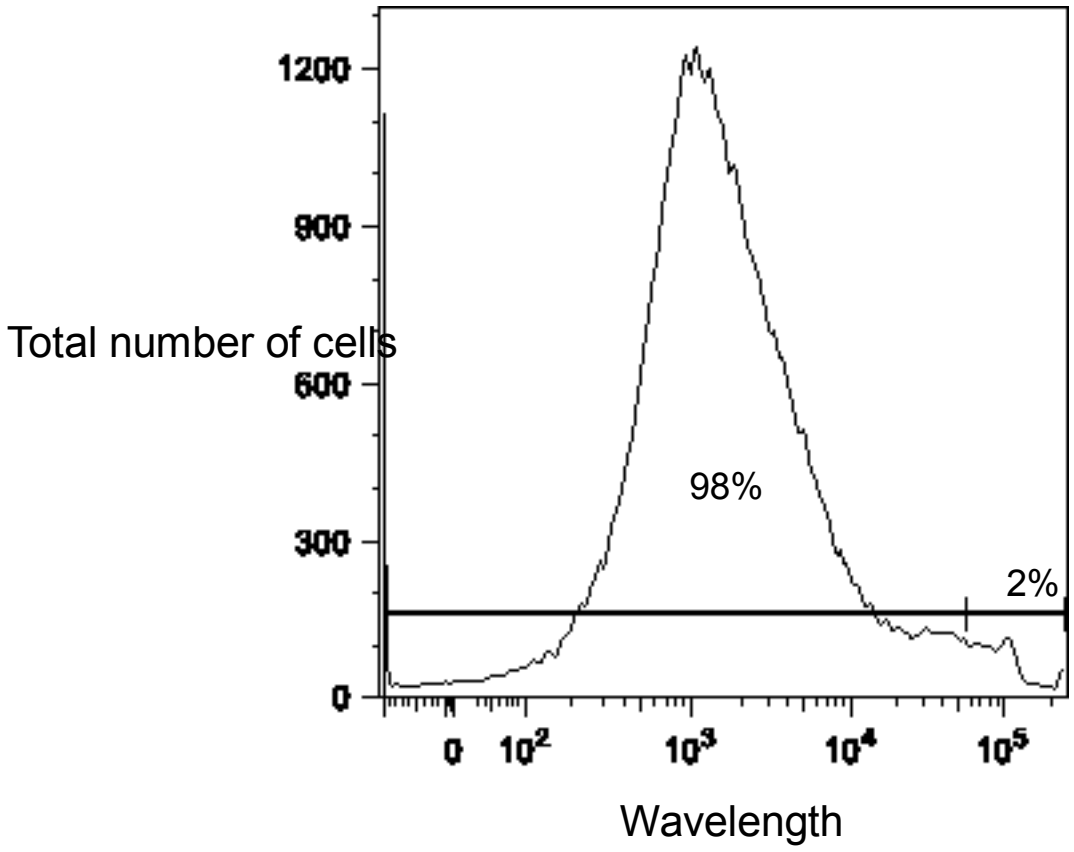


Figure 4.12 : Graph of number of cells vs. PI for typical sample (No Intervention) Second spike of higher wavelength (RHS) indicates dead bacteria which are stained with PI.

Graph showing total number of cells vs. propidium iodide (Sample B2 - Chlorhexidine)

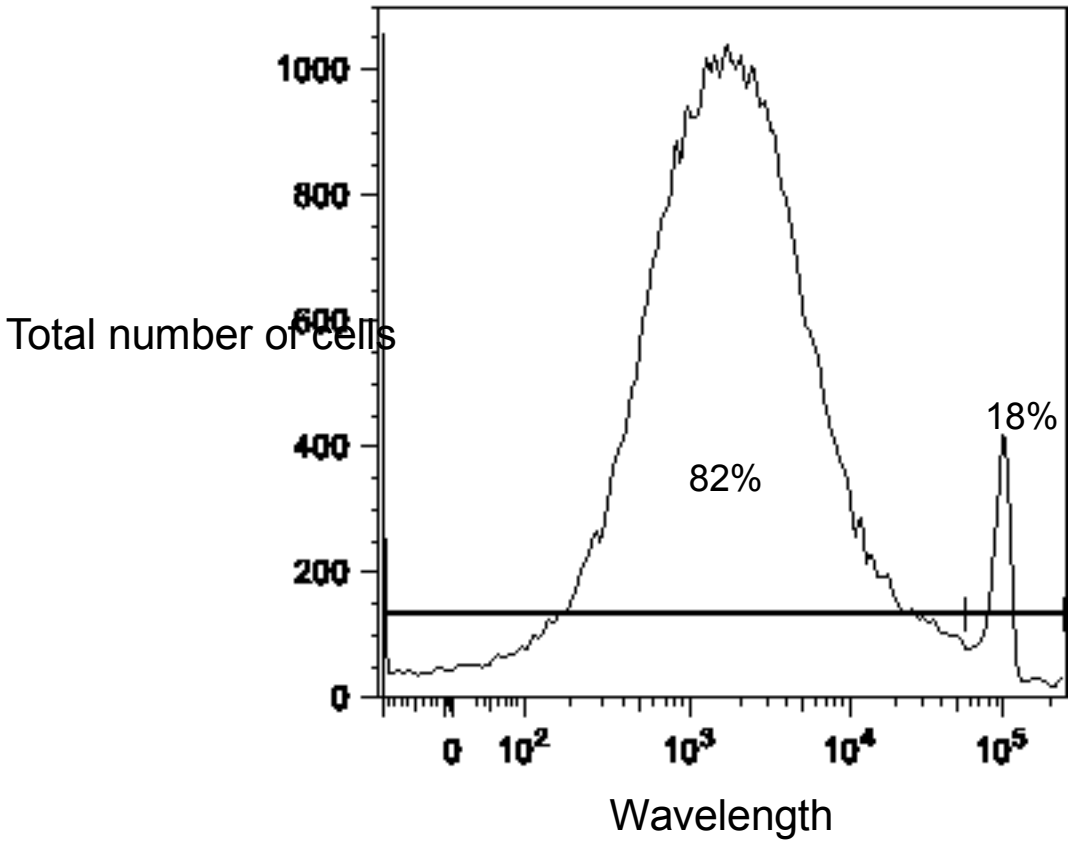


Figure 4.13 : Graph showing number of cells vs. PI for typical sample (CHX). Second spike of higher wavelength (RHS) indicates dead bacteria which are stained with PI.

Graph showing total number of cells vs. propidium iodide (Sample B3 - Fluoride)

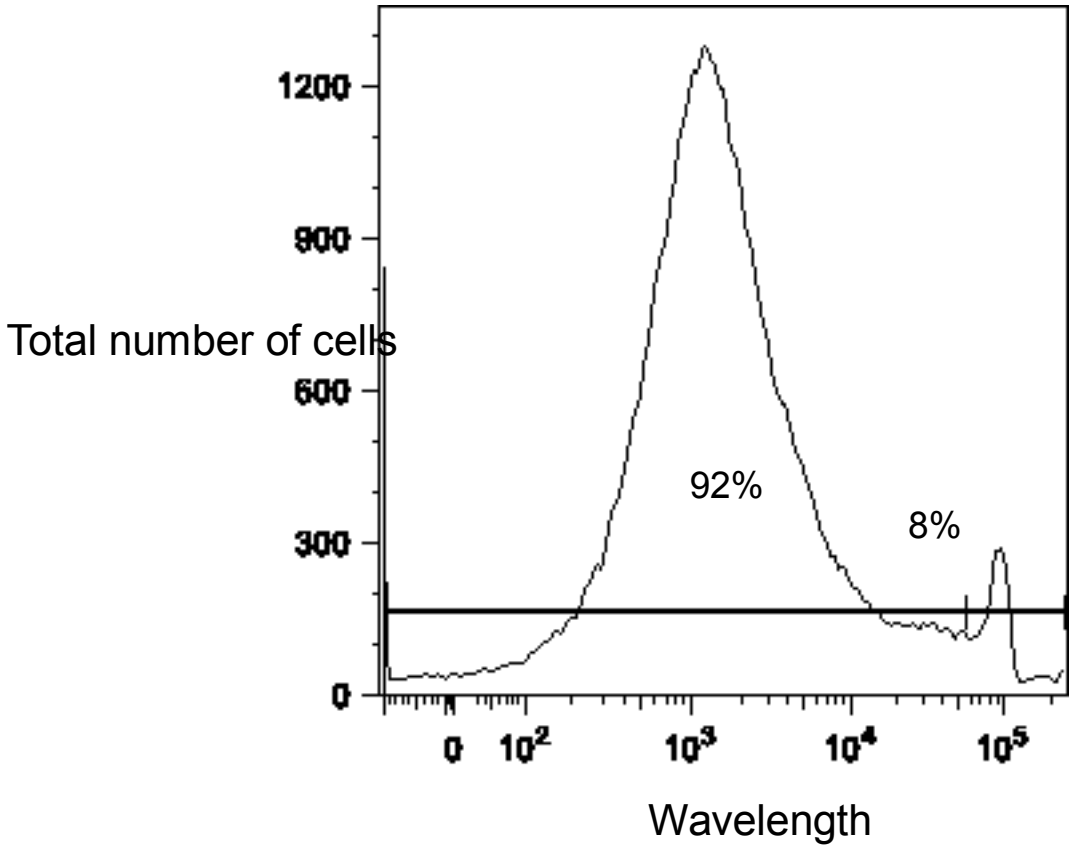


Figure 4.14 : Graph showing number of cells vs. PI for typical sample (Fluoride) Second spike of higher wavelength (RHS) indicates dead bacteria which are stained with PI.

4.6 Bacterial growth – Statistical analysis

ANOVA (Analysis of variance of means) was chosen to compare the number of bacteria (per $mm^{2)}$ roughness between the four implant groups. Prism 6 (GraphPad Software, La Jolla, CA USA) was used as the statistical program to run these analyses. Complete raw data available in Appendix D-G.

Table 4.5 : One-way ANOVA to compare means of biofilm formation

Descriptives

	Vector	Aarhus	Leone	TOMAS
Number of values	7	7	7	7
Mean Bacteria (per mm^2)	**180492***	**159485**	**72440**	**94590**
± Std. Deviation	± 69456	± 50899	± 11584	± 24535

* indicates $p<0.05$

ANOVA summary

F	9.096
P value	**0.0003**
P value summary	***
Are differences among means statistically significant? (P < 0.05)	**Yes**
R square	0.5321

Table 4.6 : Comparisons of number of biofilm grown on implant groups

Statistical test : One way ANOVA with post-hoc Tukey analysis
Dependent Variable: Number of bacteria (per mm^2)

	Mean Diff.	Significant?	Summary
Vector vs. Aarhus	21007	No	ns
Vector vs. TOMAS	108052	Yes	**
Vector vs. Leone	85903	Yes	*
Aarhus vs. TOMAS	87046	Yes	*
Aarhus vs. Leone	64896	No	ns
TOMAS vs. Leone	-22150	No	ns

* indicates $p<0.05$
** indicates $p<0.01$
ns indicates not significant

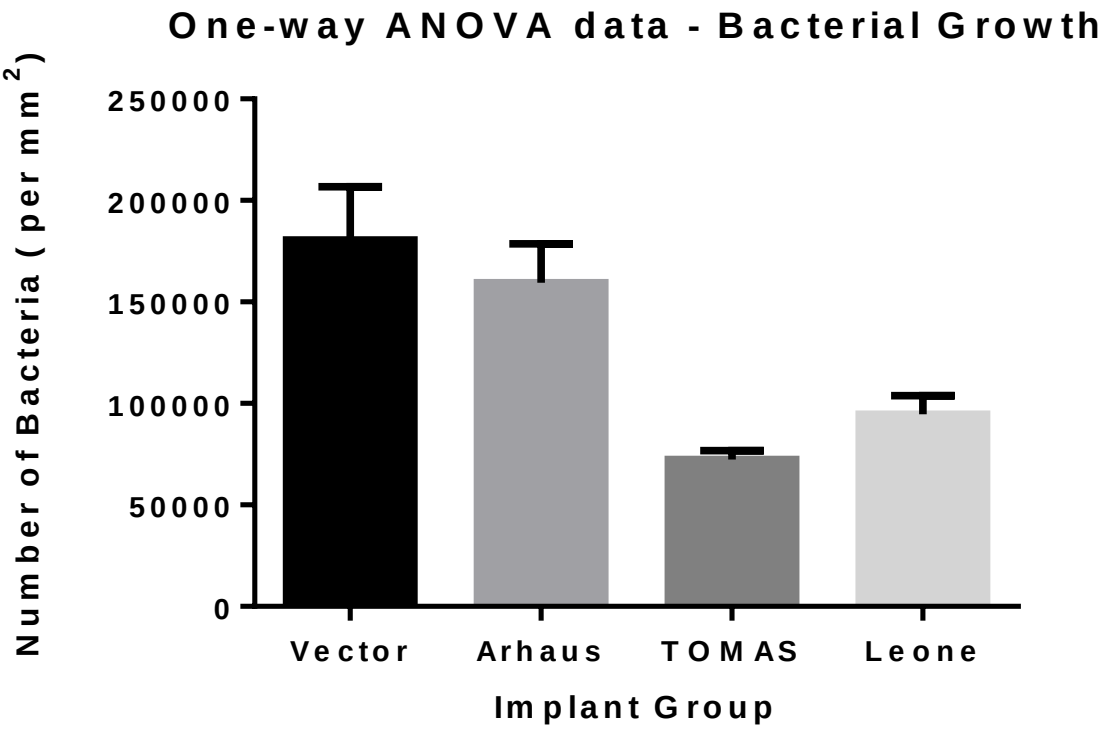


Figure 4.15 : Box plot showing the mean bacteria grown on each implant group.

The Vector implants were previously shown to be the roughest implants. They had a mean roughness (p < 0.00) of 239.4 ± 72.10 nm also had the greatest amount of biofilm aggregation (1.8 ± 0.7 x 10^5 bacteria / mm^2), (p<0.00).

This was followed by the Arhaus group: mean roughness of 127.8 ± 30.24 nm and bacterial aggregation of 1.5 ± 0.5 x 10^5 bacteria / mm^2. Next the Leone group: mean roughness of 119.9 ± 83.38 nm and bacterial aggregation of 0.95 ± 0.5 x 10^5 bacteria / mm^2. Finally, the TOMAS group: mean roughness of 127.8 ± 30.24 nm and bacterial aggregation of 0.7 ± 1.2 x 10^5 bacteria / mm^2.

Thus it appears from the groups analysed, anodized titanium alloy OMSI (Vector and Aarhus) appear roughest and accumulate the highest amount of bacteria per mm^2 followed by stainless steel (Leone). Machined titanium OMSI (TOMAS) appear to have the smoothest surface finish and have shown to accumulate the least amount of bacteria.

C

4.7 Bacterial growth – Antimicrobial agents

The proportion of dead bacteria was measured in each intervention - chlorhexidine and fluoride. Paired t tests were used to compare the efficacy of each antimicrobial agent across all the implant groups. On average, fluoride had a dead proportion of 12.8 ± 4.5% whilst chlorhexidine had a dead proportion of 36.5 ± 11%.

The antimicrobial efficacy of Chlorhexidine was greater than double than that of fluoride (p<0.0001).

Table 4.7 : Paired t-tests: Mean killing efficiency of CHX and F (Across all groups)

Descriptives

	Dead Proportion (Chlorhexidine) All implant Groups	Dead Proportion (Fluoride) All implant Groups	Difference in Dead Proportion
n	28	28	28
Mean	0.36 ± 0.11	0.13 ± 0.05	0.24* ± 0.12

* indicates p<0.05

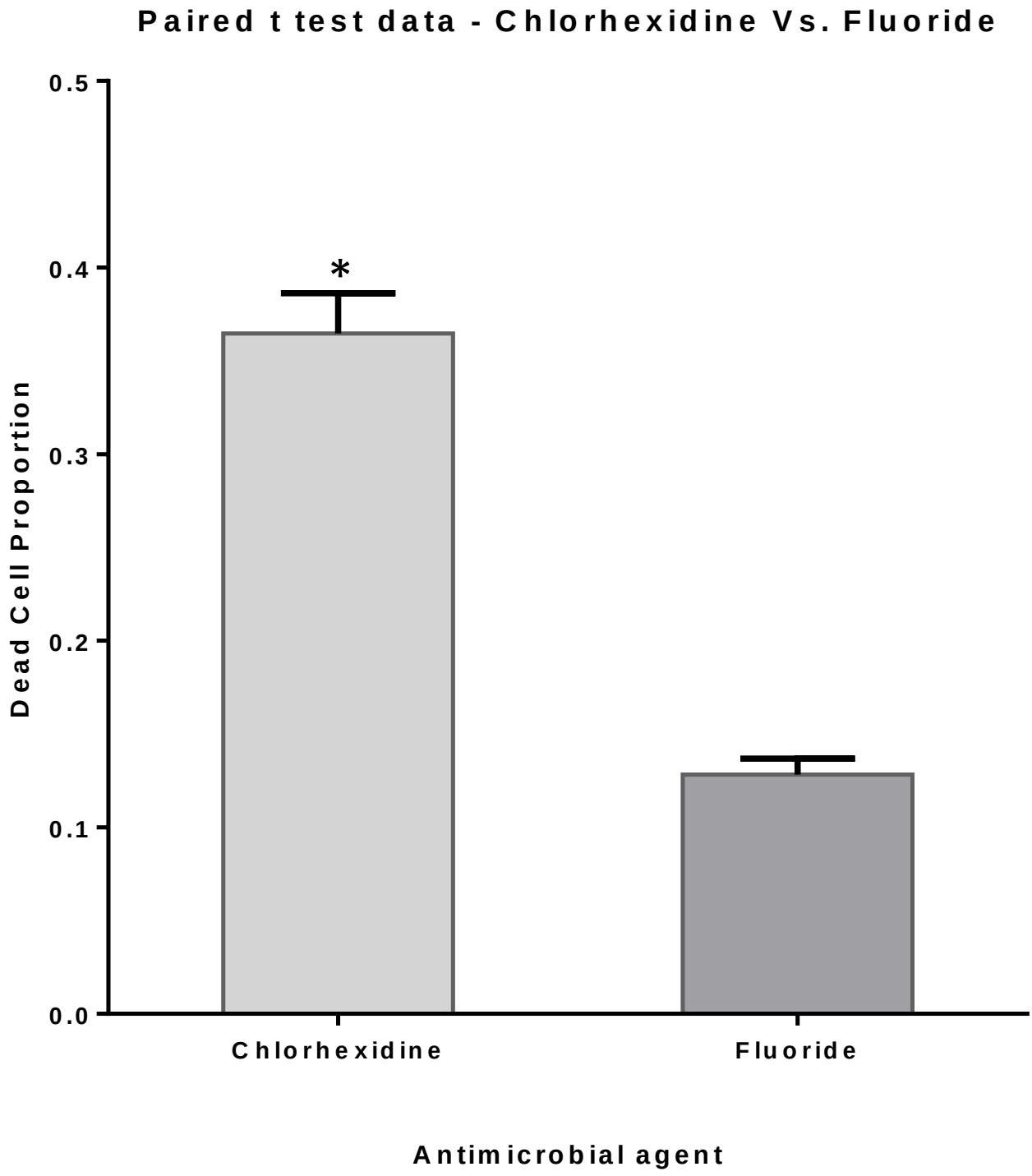


Figure 4.16 : Box plot of killing efficiency of CHX Vs. F on the surface of OMSI. Higher dead proportion indicates better killing efficacy. * indicates $p<0.05$

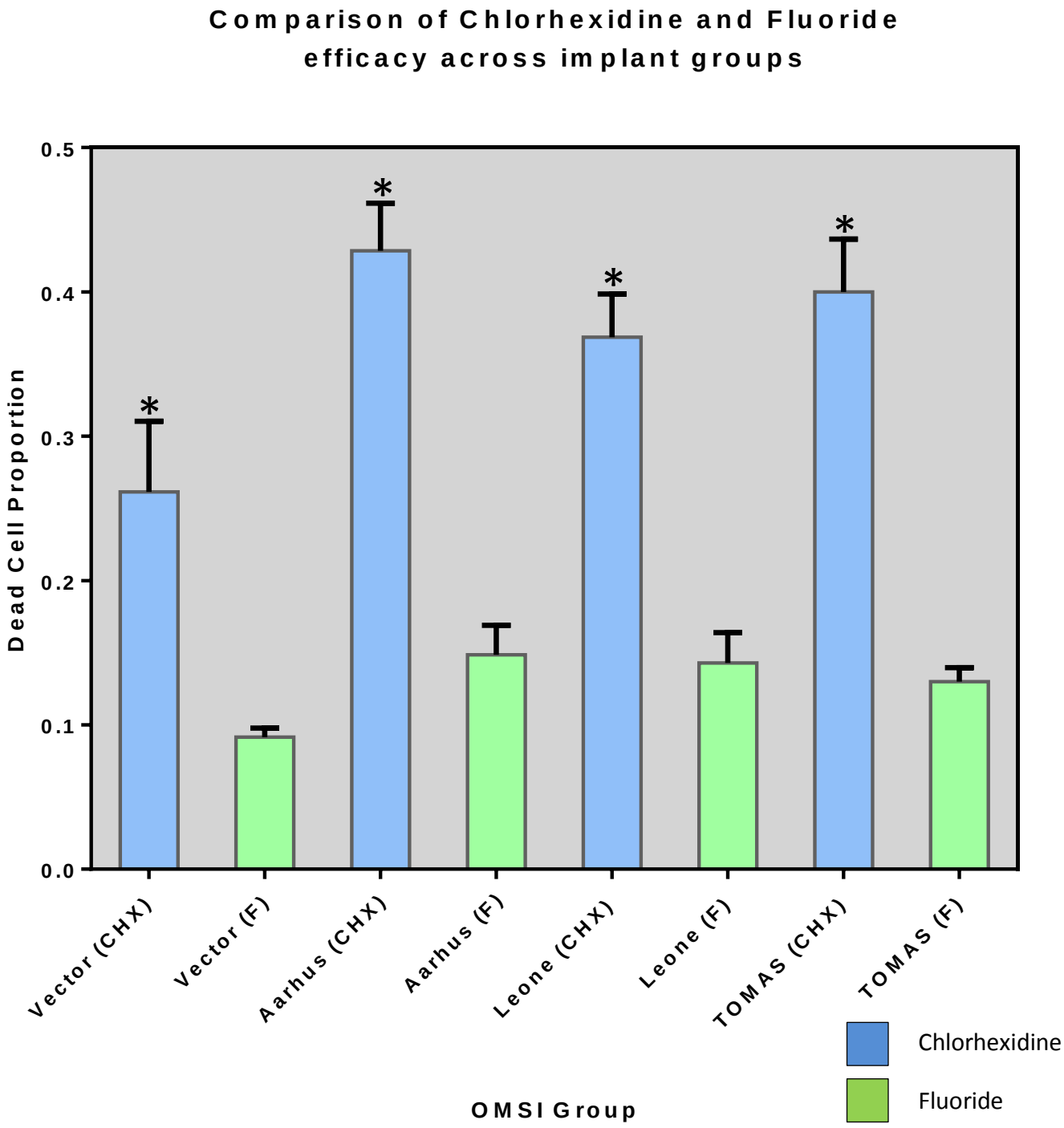


Figure 4.17 : Box plot of killing efficiency of CHX vs. F on OMSI (Across all groups) Higher dead proportion indicates better killing efficacy. * indicates $p<0.05$

It should also be noted that when the effectiveness of CHX was compared across all implant groups, the Vector group exhibited the least dead proportion of cells (highest cell viability). This was significant when compared to the Aarhus group ($p<0.05$), however not significant when compared to TOMAS or Leone.

As mentioned previously, there was significantly reduced antimicrobial effect demonstrated with F when compared with CHX ($p<0.001$). However, no significant differences could be detected when F efficacy was compared across all OMSI groups.

Chapter 5

Discussion

5.1 Discussion

It seems that the dream for an efficient and effective temporary anchorage device has almost been realised with the introduction of OMSI. Their overwhelming popularity both in clinical practice and the research field suggests that this introduction to the orthodontist's armamentarium is leading to a paradigm shift with respect to traditional principles of orthodontics. However, the relatively low and variable success rate of OMSI [211 259 200 176 187 260 94] raises questions as to the reliability of such a method of anchorage control. Furthermore these products are available to the clinical orthodontist in a myriad of materials and numerous permutations of surface finishes. There remains limited scientific data to assist clinicians in choosing an OMSI system.

One area that the literature that remains comprehensively unaddressed is biofilm formation on OMSIs. Biofilm formation has been linked with inflammation of the peri implant area which is implicated in their failure[9]. This study investigated the surface characteristics of commonly available OMSI and their effect on the development of biofilm. It also investigated the effect of antimicrobial agents on the grown biofilms.

5.1.1 Experimental design

Part of the design of the present study enabled us to elucidate the quantity and quality of biofilm formation on surface characterized OMSIs. This included OMSIs fabricated by machined Titanium alloy (Ti–6Al–4V), Anodised Titanium alloy (Ti–6Al–4V) and surgical grade stainless steel. They originated from various manufacturers, using freshly pooled human whole saliva as the source of biofilm organisms.

Human whole saliva is particularly relevant and realistic in this study as it partially mimics *in vivo* conditions [316]. These bacteria are derived from biofilms formed on hard and soft tissues in the oral cavity [317]. Furthermore human saliva provided multiple species of bacteria and was therefore more representative than a single species biofilm [318]. This was particularly relevant in an antimicrobial experiment [319].

The human saliva used contained a mixture of secretions from both major and minor salivary glands, gingival crevicular fluid, bacteria and bacterial products. It also served as a source of natural nutrition for biofilm growth. The presence of some antimicrobial substances, e.g., lysozyme, lactoferrin, lactoperoxidase, and secretory IgA may seem to compromise biofilm formation *in vitro* [320]. However, this was further thought to emulate a realistic representation of an *in vivo* situation.

It should be noted that the results of this study pertains to an *in vitro* situation. The construction of experimental designs to ascertain the success of OMSIs *in vivo* reflects a myriad of factors which may influence the outcome. For example, the difference between success and failure may be:

- Operator experience
- Site of implantation
- Local bone density
- Force vectors applied to the OMSI
- Oral hygiene
- Peri implantitis
- Material used

This study was designed with the aim of standardising as many of these variables as possible so that the effect of biofilm growth on each surface could be assessed. Hence an *in vitro* method was selected. However, it should be noted that the results of this study are to be interpreted with caution as they do not reflect a totally accurate clinical environment. An *in vivo* design involving humans or animals may have provided a more clinically relevant setting, however control of variables would have been difficult.

5.1.2 OMSI Selection

The TADs used in this study were selected because they each exhibited slightly different design features, and varying material composition and surface finish. They ranged from anodised titanium alloy (Ti-6Al-4V), un-anodised titanium alloy, to stainless steel. In addition, they were chosen from commonly available OMSI in the Australian market.

It was attempted to use similar dimensions for the OMSI (Length 10.0 mm, Diameter 2.0 mm) however the final data was corrected using the total surface area of the OMSI. To calculate the amount of bacteria grown per mm^2 on each of the implant groups, the area of each OMSI was measured. Where possible, 3D scanning of the screws along with manufacturers data was used to obtain accurate measurements of the total surface area of each OMSI. This allowed direct comparison between the different groups of OMSI.

There was little price variation between the TADs. Various other OMSI designs with different surface finishes and materials were attempted to be sourced for this study, however due to regulatory reasons they could not be provided to investigate further. This would be an interesting area for further investigation.

5.1.3 Surface roughness and surface characteristics

SEM allows high magnification and high resolution imaging of samples. A beam of high energy electrons are scanned across the surface of a sample. Interaction of the electron beam with the atoms of the sample produces a number of measurable effects which include the imaging of; surface features (morphology/topography), average chemistry (mean atomic number contrast) and cathodoluminescence (visible light). Compared to light microscopes, SEM allows for far higher magnification of images with a greatly increased depth of field (focusing range).

SEM images of the OMSIs revealed that there were numerous surface irregularities and imperfections across all of the groups. Notably, the Stainless Steel group exhibited what appeared to be significant surface pitting, as well as damage to the edges of the screw threads.

This is interesting as the stainless steel group was not packaged in a sterile container. i.e. The manufacturer instructions state that the operator should sterilise the screws before their usage. During the process of sterilisation, the delicate screws are exposed to extreme heat and pressure. It is possible that the metal may fatigue further, and begin a corrosion process [321].

This phenomenon was not found to be unique to sterilised OMSI such as the stainless steel group. The machined titanium group (TOMAS) also exhibited this characteristic pitting however it was much less common. Pitting, and striation on OMSI surfaces have been previously described in the literature [284].

The manufacture of these OMSI involves them being machined from blocks of metal. Hence the cutting portion of the milling machine may leave striations. In addition, the relative age of the blade may also influence and determine its cutting ability and cleanness. I.e. a newer blade will be sharper whilst an older blade may leave more residue and contaminants behind. The resolution and accuracy of the equipment used, as well as the technique all interplay into leaving behind evidence of metal cutting on the OMSI.

Under scanning electron microscope analysis, the Aarhus group (anodised titanium alloy) appeared to possess a superiorly smooth surface when compared to the other groups. Images in this group were without the appearance of any machining striations, and or surface irregularities.

Contrastingly, the Vector group exhibited unique surface morphology in that it's surface when imaged at the head and neck appeared roughest. This is in agreement with other Al Samak et al [322].

Despite also being an anodised titanium alloy like the Aarhus group, there were numerous striations also concluded to be originating from the manufacturing or finishing process. There are various methods of adonisation, and variation in this methodology may have led to the roughened characteristic we see here.

The results echoed that which was apparent from morphological examination. The Vector group was found to have significantly higher mean roughness than all other groups measured ($p<0.00$). There were no other statistically significant differing values of roughness detected between each of the other groups.

According to the currently available literature, commonly available dental implants have surfaces that are minimally or moderately rough. This translates to an average height deviation of 0.5–2 nm. Such a roughness has been shown to promote good osseous healing and tissue interaction. This is conducive to osseointegration of the dental implants [323 324 325 326 286].

It has also been shown through several *in vitro* studies, surface roughness of osseointegrated implant surfaces is directly proportional to biofilm formation [327] [328] [329] [330] [331] [332]. This is a characteristic not only unique to dental implant surfaces but has also been investigated regarding other dental materials such as resins, acrylics, and composites [333] [334] [335]. The literature refers to a surface roughness of 1.0 µm as moderately rough, and less than 0.5 µm as smooth [324].

It is well demonstrated in the literature that under *in vivo* conditions, smooth surfaces attract less biofilm than rough ones [336] [288]. It has been concluded that an increase in surface roughness above a threshold of 0.2 µm and/or an increase in surface free-energy facilitates biofilm formation on restorative materials. When both surface characteristics interact with each other, surface roughness was found to be dominant [288]. The range of surface roughness found of OMSI in this study echo the findings of other authors [284].

5.1.4 Surface anodising

Titanium alloy has the ability to form a protective oxide layer its surface [337]. This makes allows it to have good biocompatibility with osseous tissues. Furthermore, along with its light weight, moderate cost and workability, commercially pure titanium and alloys such as Ti–6Al–4V are often the preferred materials for many orthopaedic applications [338] [339].

With regard to Ti-6Al-4V, it has been shown that small amounts of Al and V can be leached from the alloy inside the body and minute quantities of these ions may cause local irritation to the peri implant tissues [340] [341] . This could be another potential area of investigation although beyond the scope of this research.

Certain modifications to surface topography of implant surfaces can also influence their physicochemical properties. For example, Omar [342] has illustrated that titanium implant surfaces that have undergone anodic oxidation achieve better osseointegration. This was again proven by Karmarker et al [343] who demonstrated that anodization of OMSIs may enhance their early-phase retention capability, thereby ensuring a more reliable source of absolute anchorage.

It hypothesised that this process occurs through recruitment of mesenchymal cells, the expression of genes, and factors. For example, alkaline phosphatase and osteocalcin have been implicated in bone remodelling [342].

Electro-chemical modification of the dental implant surface such as anodic oxidation can be used to change the surface morphology as well as a means of enhancing osseointegration of titanium dental implants [344] [345] [346]. Furthermore, it can also incorporate chemical elements from the electrolyte in order to improve protective properties [347] [348] [349] [349].

Whilst the modification of OMSIs surfaces with elements such as Calcium and phosphate have been shown to induce favourable cell interactions [350] [144], impurities can also be inadvertently incorporated resulting in less than ideal outcomes. Al Samak [322] in their study report the presence of phosphorous on the surface of Vector OMSI. They determine the presence of this element to be as a result of the anodising process. Chin et al [284] also report the increased presence of impurities such as carbon and nitrogen on the surface of OMSI subject to elemental analysis. This has been attributed to contamination via the manufacturing procedure, storage and sterilisation. Such contaminants have been shown to make titanium surfaces more hydrophobic, thus reducing cell attachment capacity [351] [352].

Anodic oxidation of titanium can also lead to porous surface structures which can increase surface roughness [314]. This is thought to result from the production of anatase crystals which are one of the three mineral forms of titanium dioxide. This may explain the perceived increase in surface roughness of the described implant groups.

Anatase crystallinity has also been hypothesized to reduce bacterial adhesions [353] [354]. This is thought to occur as a result of photocatalytic activity of TiO_2. However, the results of this research do not reflect the antibacterial nature of this compound. Perhaps the increased roughness is an overriding factor in bacterial aggregation.

The relationship between the nanostructure of titanium surfaces and bacterial attachment has also been discussed in the literature by Pluckett [355]. This study demonstrated that nanorough titanium (created through electron beam evaporation) is superior to nanotubular and nanotextured titanium (created through adonization). This kind of titanium treatment has been shown to enhance osteoblast adhesion whilst discouraging the growth of unwanted cells.

Del Curto et al. [353] also discussed a method of heat treating anodized titanium to modify the titanium oxide film. This has shown to decease bacterial adhesion, however further work is still needed in this field.

5.1.5 Biofilm growth on implant surfaces.

The microflora of both healthy and diseased oral implants reflects that of teeth in similar clinical states [356] [281]. It is well researched that though interactions with the acquired salivary pellicle, microbial colonization is initiated by the adhesion of pioneer species, such as Streptococcus aureus, Streptococcus sanguinis and Actinomyces naeslundii. These microorganisms are all thought to be commensals of the oral environment [357].

It should be noted however that exposure of an implant surface that has been optimized to encourage osseointegration to the oral environment generally results in deleterious consequences [358]. As the modified implant surfaces are optimized for tissue healing, bacteria take advantage of such surfaces and adhere . Biofilm growth is subsequently promoted through the attraction of further secondary and tertiary colonisers. Layered structures develop which allow microbial communities to live in close physical contact.

Interactions between microorganisms facilitate the ability of the microorganisms to survive under environmentally stressful conditions as well as in the presence of antimicrobial agents [359] [360] [361].

Interactions between biomaterial surfaces and bacteria are thought to be based on a variety of forces including electrostatic, Lifshitz–Van der Waals and hydrophobic forces, as well as various specific receptor–ligand interactions [362] [363] [364].

In the case of bacteria, the negative charge of most metal biomaterial surfaces at physiological pH is believed to initially repel negatively charged bacteria [365] [366] [367]. This effect is, however limited in time since it is known that surfaces of biomaterial implants become rapidly coated with host plasma constituents, including extracellular matrix proteins and other biomolecules once they are implanted in the body [368] [369]. In addition, not all pathogens are negatively charged at physiological pH.

Furthermore, in the case of anodic oxide layers, it has been shown that the formation conditions of the anodic oxide layer and composition, concentration and pH value of the solution which is in contact with the anodized surfaces can influence on the charge and hydrophilic/hydrophobic characteristics of the surface [370].

5.1.6 The future of biomaterial coatings

Further research into coatings for biomaterials is warranted. Currently, most coatings for OMSI are mono-functional in that they are designed primarily to discourage biofilm formation, or enhance osseous contact One approach that has been suggested has been to include a bi-functional coating containing anti-adhesive functionalities, such as a polyethylene glycol polymer brush to discourage biofilm formation, while at the same time possessing functionalities like arginine-glycine-aspartic acid sequences to support tissue integration [371] [372]. Harris et al. demonstrated an example of differential adhesion on titanium oxide surfaces coated with Poly(l-lysine)-grafted-poly(ethylene glycol) (PLL-g-PEG). In their in vitro study, they demonstrated that surfaces coated with PLL-g-PEG/PEG-RGD have the ability to attach cells such as fibroblasts and osteoblasts while showing reduced Streptococcus aureus adhesion, resulting in a selective bio-interaction pattern [373].

Such features may improve the biocompatibility and retention of OMSI [354]. However it should be noted that their implementation is also likely to increase the costs of OMSI.

5.1.7 Choice of Antimicrobial Agents

The chemo manipulation of biofilms around OMSIs remains an important part of their maintenance. Mechanical debridement of the area can cause trauma, and localised inflammation which may lead to negative outcomes for the OMSI such as reduced stability and eventual loss. Patients should be instructed on light, mechanical debridement with a soft toothbrush. However commonly available antimicrobial agents may prove more effective when used in unison with mechanical debridement.

This study investigated the immediate *in vitro* bactericidal effects of commercially available chlorhexidine and fluoride mouth rinses in the biofilm mode of growth on micro-implant surfaces. The exposure time of 1 minute was designed to simulate a single rinse of chlorhexidine or fluoride rinse used in a clinical situation.

The initial cell viability of biofilms of 82.4± 15.8% was in agreement with past studies into the viability of similar biofilms [284] [374]. It has been revealed that with increasing time, viability decreases [374]. This has been hypothesized to occur as a result of the antimicrobial effect of the saliva on the biofilm [298].

Indeed the effective antimicrobial ability of both agents was demonstrated in this experiment. With chlorhexidine demonstrating greater than twice as effective at killing bacteria than fluoride ($p<0.0001$).

The effectiveness of these antimicrobial agents is in agreement with numerous other studies using either *in vitro* biofilm models or biofilm scrapings from intra-oral surfaces, which reported biofilm viability ranging from 7% to 23% for chlorhexidine and 14% to 54% for fluoride, respectively [374] [297] [375].

The exposure of bacteria to the antimicrobial agents in this experiment was only 1 minute. Thus with longer exposure it is hypothesised that the antimicrobial effect would be greater.

Furthermore, whilst commercially available mouth rinses with set concentrations were selected in this study, it is also anticipated that increasing concentration of antimicrobial agent will prove more effective.

Chlorhexidine is unique in that the cationic nature of the molecule allows binding to surfaces. This phenomenon is commonly referred to as substantively. Previous studies have shown that chlorhexidine adsorption is proportional to increased roughness [376] [377]. However, as discussed, increased roughness may also increase bacterial aggregation.

Crystalline titanium dioxide most commonly exists in one of the two structures, anatase and rutile, and both of these have been observed on commercially available dental implants. Barbour et al. [378] demonstrated that there is a preferential adsorption of chlorhexidine to anatase compared with rutile.

Comparatively, fluoride has been shown by various studies to induce a spectrum of effects on titanium alloys as it has been shown that their action is relatively aggressive on the protective layer of titanium and titanium alloys [379] [380] [381]. The presence of F ions may possibly start a localized corrosive degradation by pitting and crevice corrosion processes [380]. This can in turn lead to morphological variations, such as increased roughness [382]. In the case of other orthodontic materials such as NiTi wires, fracture of the wire under masticatory load has been documented [383]. It should also be noted that the formation of biofilms on OMSIs leads to a localised reduction in pH and the depletion of oxygen. Both of these processes may accelerate the deterioration of the protective titanium oxide layer on such devices [384]. Thus according to the results of this study, such an effect has a positive feedback on the growth of biofilms.

Furthermore, the corrosion of Titanium OMSIs facilitated by low pH and F ions may lead to leaching of metallic ions into the adjacent tissues. This has been shown to cause localised inflammation [385] [386].

However this only occurs over a significant period of time, and due to the temporary nature of OMSIs, it is thought that any deleterious effect may be clinically insignificant.

Although beyond the scope of this study, it would be interesting to conduct investigation into the change in surface properties, of the OMSI after a period of exposure to F. In addition, whilst not investigated here, the anti caries benefit of F is unrivalled and this its application may be linked to other needs [387] [388] [389].

With respect to the investigated antimicrobial agents, the current literature as well as the finding of this study suggests that chlorhexidine remain a pertinent choice for clinicians when recommending an antimicrobial agent to effectively clean OMSI *in situ*. Contrastingly F is not as effective an antimicrobial agent, although its anti-cariogenic properties may address other needs in the susceptible individual.

Chapter 6

Conclusions

6.1 Conclusions

The results of the present study provide additional data which may be helpful in identifying surface characteristics of OMSI and how they affect biofilm growth.

The study fulfilled the aims and objectives set within the limitation of an *in vitro* model. All of the null hypotheses were proven incorrect with statistically significant differences found in;

- The surface roughness between implant groups.
- The amount of growth of biofilm between implant groups.
- The killing efficacy of antimicrobial agents.

Based on the results of this study, there appears to be a relationship between increased surface roughness and increased biofilm growth. Anodized surfaces on titanium alloy appear to possess the roughest surface, whilst stainless steel and machined uncoated titanium are amongst the smoothest. This may be a factor in the operator choosing an appropriate OMSI system for use in their patients to limit the amount of biofilm growth.

Furthermore, when selecting an antimicrobial agent to assist patients in keeping OMSIs plaque free, chlorhexidine remains the best choice when compared to fluoride. It provides greater than twice the efficacy of fluoride, and has increased substantively.

However, caution must be exercised in extrapolating the findings of this *in vitro* study to an *in vivo* situation. Further research is required to investigate the clinical implications of such surface roughness on biofilm formation on these implants. Perhaps the use of an animal model within the framework of a randomized clinical trial would provide the next step of evidence on the hierarchical pyramid.

Chapter 7

Appendices

7.1 Appendix A: Ethics approval

This administrative form has been removed

7.2 Appendix B: Ethics information form

This administrative form has been removed

7.3 Appendix B: Ethics consent form

This administrative form has been removed

7.4 Appendix C: Roughness data

	Aarhus	Vector	TOMAS	Leone
1	128.38	239.651	88.9748	54.1807
2	133.035	232.285	104.601	62.4042
3	145.246	250.836	114.844	61.0109
4	130.352	269.771	88.9748	102.995
5	139.145	221.456	86.3039	91.4909
6	96.0455	377.972	92.8152	92.8362
7	115.489	270.214	127.424	27.9944
8	110.195	209.303	236.989	112.465
9	84.5752	151.289	196.785	91.9291
10	94.2063	129.301	59.152	91.8346
11	101.433	176.195	66.5125	378.173
12	160.132	299.414	50.6577	227.013
13	138.499	214.119	86.8262	132.307
14	207.201	151.287	120.928	170.076
15	112.864	259.568	105.534	99.2856
16	147.737	377.972	66.5125	123.153

Table 7.1 : RAW Data used to calculate mean surface roughness of implant groups. All roughness listed in nanometres (nm)

7.5 Appendix D: Biofilm data (Vector)

	Sample	Total bacteria in sample / mm^2	Total live in sample / mm^2	Total dead in sample / mm^2	DEAD Proportion	LIVE Proportion
1	V1	142514.21	140960.65	1553.56	0.01	0.99
2	V1	184178.53	176907.11	7271.42	0.04	0.96
3	V1	146277.97	135453.40	10824.57	0.07	0.93
4	V1	196215.83	190190.20	6025.63	0.03	0.97
5	V1	146699.08	139832.19	6866.89	0.05	0.95
6	V1	120869.80	114584.57	6285.23	0.05	0.95
7	V1	326690.20	320547.20	6143.00	0.02	0.98
8	V2	10515.06	9964.27	550.79	0.05	0.95
9	V2	349534.52	223117.18	126417.34	0.36	0.64
10	V2	194217.18	143068.38	51148.80	0.26	0.74
11	V2	208767.35	137969.25	70798.10	0.34	0.66
12	V2	166550.57	97059.14	69491.43	0.42	0.58
13	V2	323323.83	279079.51	44244.31	0.14	0.86
14	V2	326141.92	241976.26	84165.66	0.26	0.74
15	V3	219438.06	198015.62	21422.44	0.10	0.90
16	V3	204079.62	188037.15	16042.47	0.08	0.92
17	V3	155738.75	138208.61	17530.14	0.11	0.89
18	V3	121115.08	108832.98	12282.09	0.10	0.90
19	V3	316788.69	297848.57	18940.11	0.06	0.94
20	V3	257072.55	232303.96	24768.59	0.10	0.90
21	V3	281653.04	255021.85	26631.19	0.09	0.91

Table 7.2 : RAW data used to calculate the mean biofilm grown for Vector . V1: No intervention, V2: Chlorhexidine, V3: Fluoride.

7.6 Appendix E: Biofilm data (Aarhus)

	Sample	Total bacteria in sample / mm^2	Total live in sample / mm^2	Total dead in sample / mm^2	DEAD Proportion	LIVE Proportion
22	A1	133343.17	129540.99	3802.18	0.03	0.97
23	A1	112706.42	107430.70	5275.72	0.05	0.95
24	A1	100063.65	95508.48	4555.17	0.05	0.95
25	A1	229995.70	225625.78	4369.92	0.02	0.98
26	A1	223361.54	215342.06	8019.48	0.04	0.96
27	A1	165079.80	157501.88	7577.92	0.05	0.95
28	A1	151846.94	147139.68	4707.26	0.03	0.97
29	A2	273154.87	133971.27	139183.61	0.51	0.49
30	A2	191479.62	94497.72	96981.89	0.51	0.49
31	A2	360439.94	208903.63	151536.31	0.42	0.58
32	A2	308804.16	222584.04	86220.12	0.28	0.72
33	A2	344057.30	201186.05	142871.25	0.42	0.58
34	A2	219471.54	140768.50	78703.05	0.36	0.64
35	A2	290537.86	145804.41	144733.45	0.50	0.50
36	A3	123113.80	101711.92	21401.88	0.17	0.83
37	A3	287741.60	252113.99	35627.62	0.12	0.88
38	A3	194874.05	162071.80	32802.25	0.17	0.83
39	A3	133527.74	103471.68	30056.06	0.23	0.77
40	A3	313400.66	259477.91	53922.75	0.17	0.83
41	A3	146777.03	128719.29	18057.74	0.12	0.88
42	A3	11366.62	10659.25	707.38	0.06	0.94

Table 7.3 : RAW data used to calculate the mean biofilm grown for Aarhus. A1: No intervention, A2: Chlorhexidine, A3: Fluoride.

7.7 Appendix F: Biofilm data (TOMAS)

	Sample	Total bacteria in sample / mm^2	Total live in sample / mm^2	Total dead in sample / mm^2	DEAD Proportion	LIVE Proportion
43	T1	85740.37	84600.36	1140.00	0.01	0.99
44	T1	83257.90	80859.59	2398.31	0.03	0.97
45	T1	73947.71	71306.98	2640.73	0.04	0.96
46	T1	64337.20	62670.70	1666.50	0.03	0.97
47	T1	57085.09	55098.13	1986.96	0.03	0.97
48	T1	61227.77	59292.20	1935.57	0.03	0.97
49	T1	81482.44	80268.23	1214.21	0.01	0.99
50	T2	109510.45	60895.41	48615.04	0.44	0.56
51	T2	96357.68	51943.23	44414.44	0.46	0.54
52	T2	189380.46	109057.80	80322.66	0.42	0.58
53	T2	59022.79	39121.16	19901.63	0.34	0.66
54	T2	139591.47	93060.98	46530.49	0.33	0.67
55	T2	130312.23	99889.14	30423.09	0.23	0.77
56	T2	64744.66	41239.72	23504.94	0.36	0.64
57	T3	110988.04	82997.44	27990.60	0.25	0.75
58	T3	76656.42	68201.16	8455.27	0.11	0.89
59	T3	87440.71	76500.78	10939.93	0.13	0.87
60	T3	64002.78	55530.14	8472.64	0.13	0.87
61	T3	116880.27	108926.22	7954.04	0.07	0.93
62	T3	71029.83	60703.35	10326.47	0.15	0.85
63	T3	88133.85	74060.12	14073.73	0.16	0.84

Table 7.4 : RAW data used to calculate the mean biofilm grown for TOMAS. T1: No intervention, T2: Chlorhexidine, T3: Fluoride.

7.8 Appendix G: Biofilm data (Leone)

	Sample	Total bacteria in sample / mm^2	Total live in sample / mm^2	Total dead in sample / mm^2	DEAD Proportion	LIVE Proportion
64	L1	71030.09	69339.24	1690.85	0.02	0.98
65	L1	100804.50	98043.56	2760.94	0.03	0.97
66	L1	63210.00	61275.00	1935.00	0.03	0.97
67	L1	123788.49	121907.28	1881.21	0.02	0.98
68	L1	107100.00	103285.71	3814.29	0.04	0.96
69	L1	120454.99	116491.62	3963.37	0.03	0.97
70	L1	75739.88	73633.89	2105.99	0.03	0.97
71	L2	136160.37	76814.55	59345.82	0.44	0.56
72	L2	175490.48	115688.45	59802.03	0.34	0.66
73	L2	168476.24	123835.08	44641.16	0.26	0.74
74	L2	148626.72	83847.41	64779.31	0.44	0.56
75	L2	126377.97	55066.78	71311.19	0.56	0.44
76	L2	78958.67	51823.28	27135.39	0.34	0.66
77	L2	96716.41	55920.51	40795.90	0.42	0.58
78	L3	80206.48	68545.89	11660.60	0.15	0.85
79	L3	139047.67	123654.24	15393.43	0.11	0.89
80	L3	144911.04	119492.42	25418.62	0.18	0.82
81	L3	140924.86	123475.82	17449.05	0.12	0.88
82	L3	78685.17	70270.03	8415.14	0.11	0.89
83	L3	87762.38	77069.31	10693.07	0.12	0.88
84	L3	109397.47	96068.35	13329.11	0.12	0.88

Table 7.5 : RAW data used to calculate the mean biofilm grown for Leone. L1: No intervention, L2: Chlorhexidine, L3: Fluoride.

Chapter 8

References

8.1 List of references

1. Orthodontics A. Sure smile photo;Online. http://abcorthodontics.com.au/files/8813/4749/9345/suresmile1.jpeg

2. Melsen B, Verna C. A rational approach to orthodontic anchorage. Progress in Orthodontics 2000;1(1):10-22.

3. Brånemark BK. 2010 2012]

4. De Clerck EE, Swennen GR. Success rate of miniplate anchorage for bone anchored maxillary protraction. The Angle Orthodontist 2011;81(6):1010-3.

5. Tinsley D, O'Dwyer JJ, Benson PE et al. Orthodontic palatal implants: clinical technique. Journal of Orthodontics 2004;31(1):3-8.

6. Brettin BT, Grosland NM, Qian F et al. Bicortical vs monocortical orthodontic skeletal anchorage. American Journal of Orthodontics and Dentofacial Orthopedics 2008;134(5):625-35.

7. Block MS, Hoffman DR. A new device for absolute anchorage for orthodontics. Am J Orthod Dentofacial Orthop 1995;107(3):251-8.

8. Jaggi N BR, Mehan P. Implantable Devices: A Review of Current Treatment Modalities. Int J Oral Implantol Clin Res 2012;;3(1):31-35.

9. Miyawaki S, Koyama I, Inoue M et al. Factors associated with the stability of titanium screws placed in the posterior region for orthodontic anchorage. Am J Orthod Dentofacial Orthop 2003;124(4):373-8.

10. Subramani K, Jung RE, Molenberg A et al. Biofilm on dental implants: a review of the literature. Int J Oral Maxillofac Implants 2009;24(4):616.

11. Corruccini RS, Pacciani E. "Orthodontistry" and dental occlusion in Etruscans. Angle Orthod 1989;59(1):61-4.

12. Proffit WR, Fields HW, Sarver DM. Contemporary Orthodontics: Mosby Elsevier; 2007.

13. Wahl N. Orthodontics in 3 millennia. Chapter 1: Antiquity to the mid-19th century. Am J Orthod Dentofacial Orthop 2005;127(2):255-9.

14. Bonnick AM, Nalbandian M, Siewe MS. Technological Advances in Nontraditional Orthodontics. Dental Clinics of North America 2011;55(3):571-84.

15. Misch CE. Contemporary Implant Dentistry - E-Book: Elsevier Health Sciences; 2007.

16. Branemark PI. Osseointegration and its experimental background. J Prosthet Dent 1983;50(3):399-410.

17. Branemark PI, Adell R, Breine U et al. Intra-osseous anchorage of dental prostheses. I. Experimental studies. Scand J Plast Reconstr Surg 1969;3(2):81-100.

18. Adell R, Lekholm U, Rockler B et al. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg 1981;10(6):387-416.

19. Adell R, Eriksson B, Lekholm U et al. Long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. Int J Oral Maxillofac Implants 1990;5(4):347.

20. Jung RE, Pjetursson BE, Glauser R et al. A systematic review of the 5-year survival and complication rates of implant-supported single crowns. Clin Oral Implants Res 2007;19(2):119-30.

21. Pjetursson BE, Tan K, Lang NP et al. A systematic review of the survival and complication rates of fixed partial dentures (FPDs) after an observation period of at least 5 years. Clin Oral Implants Res 2004;15(6):625-42.

22. Levin L, Sadet P, Grossmann Y. A retrospective evaluation of 1,387 single-tooth implants: a 6-year follow-up. Journal of periodontology 2006;77(12):2080-3.

23. Pjetursson BE, Brägger U, Lang NP et al. Comparison of survival and complication rates of tooth-supported fixed dental prostheses (FDPs) and implant-supported FDPs and single crowns (SCs). Clin Oral Implants Res 2007;18:97-113.

24. Brägger U, Karoussis I, Persson R et al. Technical and biological complications/failures with single crowns and fixed partial dentures on implants: a 10-year prospective cohort study. Clin Oral Implants Res 2005;16(3):326-34.

25. Jemt T. Fixed implant-supported prostheses in the edentulous maxilla. A five-year follow-up report. Clin Oral Implants Res 1994;5(3):142-7.

26. Maló P, Rangert B, Nobre M. All-on-4 Immediate-Function Concept with Brånemark System® Implants for Completely Edentulous Maxillae: A 1-Year Retrospective Clinical Study. Clin Implant Dent Relat Res 2006;7(s1):s88-s94.

27. Daskalogiannakis J. Glossary of orthodontic terms: Quintessence Books; 2000.

28. Angle EH. Treatment of malocculsion of the teeth: The S. S. White dental manufacturing company; 1907.

29. Ottofy L. Standard dental dictionary: Laird & Lee, inc.; 1923.

30. Moyers RE. Handbook of Orthodontics for the Student and General Practitioner: Year Book Medical Publishers; 1970.

31. Gianelly AA, Goldman HM. Biologic basis of orthodontics: Lea & Febiger; 1971.

32. Marcotte MR. Biomechanics in orthodontics: B.C. Decker; 1990.

33. Burstone CJ, Van Steenbergen E, Hanley KJ. Modern edgewise mechanics and the segmented arch technique: Department of Orthodontics, University of Connecticut, School of Dental Medicine; 1995.

34. Kokich VG, Shapiro PA, Oswald R et al. Ankylosed teeth as abutments for maxillary protraction: a case report. Am J Orthod 1985;88(4):303-7.

35. Cope JB. Temporary Anchorage Devices in Orthodontics: W.B. Saunders; 2005.

36. Graber TM, Swain BF. Current orthodontic concepts and techniques: Saunders; 1975.

37. Oppenheim A. Biologic orthodontic therapy and reality. The Angle Orthodontist 1936;6(2):69-116.

38. Kloehn SJ. Guiding alveolar growth and eruption of teeth to reduce treatment time and produce a more balanced denture and face. The Angle Orthodontist 1947;17(1):10-33.

39. Braun S, Lee KG, Legan HL. A reexamination of various extraoral appliances in light of recent research findings. The Angle Orthodontist 1999;69(1):81-4.

40. Braun S. Extraoral appliances: a twenty-first century update. American Journal of Orthodontics and Dentofacial Orthopedics 2004;125(5):624-9.

41. Rock W. The use of extra-oral traction with removable appliances. British Dental Journal 1990;168(10):403-7.

42. Cole WA. Accuracy of patient reporting as an indication of headgear compliance. American journal of orthodontics and dentofacial orthopedics: official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics 2002;121(4):419.

43. Cureton SL, Regennitter FJ, Yancey JM. Clinical versus quantitative assessment of headgear compliance. American Journal of Orthodontics and Dentofacial Orthopedics 1993;104(3):277-84.

44. Ağar U, Doruk C, Biçakçi AA et al. The role of psycho-social factors in headgear compliance. The European Journal of Orthodontics 2005;27(3):263-7.

45. Egolf RJ, BeGole EA, Upshaw HS. Factors associated with orthodontic patient compliance with intraoral elastic and headgear wear. American Journal of Orthodontics and Dentofacial Orthopedics 1990;97(4):336-48.

46. Cureton SL, Regennitter FJ, Yancey JM. Clinical versus quantitative assessment of headgear compliance. Am J Orthod Dentofacial Orthop 1993;104(3):277-84.

47. Bartsch A, Witt E, Sahm G et al. Correlates of objective patient compliance with removable appliance wear. Am J Orthod Dentofacial Orthop 1993;104(4):378-86.

48. Samuels RHA, Brezniak N. Orthodontic facebows: safety issues and current management. Journal of Orthodontics 2002;29(2):101-8.

49. Samuels RH, Brezniak N. Orthodontic facebows: safety issues and current management. J Orthod 2002;29(2):101-7.

50. Yamaguchi M, Inami T, Ito K et al. Mini-Implants in the Anchorage Armamentarium: New Paradigms in the Orthodontics. Int J Biomater 2012;2012.

51. Papadopoulos MA, Tarawneh F. The use of miniscrew implants for temporary skeletal anchorage in orthodontics: a comprehensive review. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 2007;103(5):e6-e15.

52. Gainsforth BL, Higley LB. A study of orthodontic anchorage possibilities in basal bone. American Journal of Orthodontics and Oral Surgery 1945;31(8):406-17.

53. Creekmore TD, Eklund MK. The possibility of skeletal anchorage. J Clin Orthod 1983;17(4):266-9.

54. Linkow LI. The endosseous blade implant and its use in orthodontics. Int J Orthod 1969;7(4):149-54.

55. Roberts WE, Smith RK, Zilberman Y et al. Osseous adaptation to continuous loading of rigid endosseous implants. Am J Orthod 1984;86(2):95-111.

56. Douglass JB, Killiany DM. Dental implants used as orthodontic anchorage. J Oral Implantol 1987;13(1):28-38.

57. Fernandez Valeron J, Fernandez Velazquez J. Implants in the orthodontic and prosthetic rehabilitation of an adult patient: a case report. Int J Oral Maxillofac Implants 1996;11(4):534-8.

58. Haanaes HR, Stenvik A, Beyer-Olsen ES et al. The efficacy of two-stage titanium implants as orthodontic anchorage in the preprosthodontic correction of third molars in adults--a report of three cases. Eur J Orthod 1991;13(4):287-92.

59. Southard TE, Buckley MJ, Spivey JD et al. Intrusion anchorage potential of teeth versus rigid endosseous implants: a clinical and radiographic evaluation. Am J Orthod Dentofacial Orthop 1995;107(2):115-20.

60. Armbruster PC, Block MS. Onplant-supported orthodontic anchorage. Atlas Oral Maxillofac Surg Clin North Am 2001;9(1):53-74.

61. Janssens F, Swennen G, Dujardin T et al. Use of an onplant as orthodontic anchorage. Am J Orthod Dentofacial Orthop 2002;122(5):566-70.

62. Hong H, Ngan P, Han G et al. Use of onplants as stable anchorage for facemask treatment: a case report. Angle Orthod 2005;75(3):453-60.

63. Chen X, Chen G, He H et al. Osseointegration and biomechanical properties of the onplant system. Am J Orthod Dentofacial Orthop 2007;132(3):278 e1-6.

64. Heuberer S, Dvorak G, Zauza K et al. The use of onplants and implants in children with severe oligodontia: a retrospective evaluation. Clin Oral Implants Res 2012;23(7):827-31.

65. Thebault B, Bedhet N, Behaghel M et al. The benefits of using anchorage miniplates: are they compatible with everyday orthodontic practice? Int Orthod 2011;9(4):353-87.

66. Umemori M, Sugawara J, Mitani H et al. Skeletal anchorage system for open-bite correction. Am J Orthod Dentofacial Orthop 1999;115(2):166-74.

67. Yamaguchi M, Inami T, Ito K et al. Mini-implants in the anchorage armamentarium: new paradigms in the orthodontics. Int J Biomater 2012;2012:394121.

68. Choi BH, Zhu SJ, Kim YH. A clinical evaluation of titanium miniplates as anchors for orthodontic treatment. Am J Orthod Dentofacial Orthop 2005;128(3):382-4.

69. Sherwood KH, Burch JG. Skeletally based miniplate supported orthodontic anchorage. J Oral Maxillofac Surg 2005;63(2):279-84.

70. Melsen B, Petersen JK, Costa A. Zygoma ligatures: an alternative form of maxillary anchorage. J Clin Orthod 1998;32(3):154-8.

71. Wehrbein H, Glatzmaier J, Mundwiller U et al. The Orthosystem--a new implant system for orthodontic anchorage in the palate. J Orofac Orthop 1996;57(3):142-53.

72. Nienkemper M, Wilmes B, Renger S et al. [Improvement of mini-implant stability in orthodontics]. Orthod Fr 2012;83(3):201-7.

73. Costa A, Raffainl M, Melsen B. Miniscrews as orthodontic anchorage: a preliminary report. Int J Adult Orthodon Orthognath Surg 1998;13(3):201-9.

74. Rossouw PE, Buschang PH. Temporary orthodontic anchorage devices for improving occlusion. Orthod Craniofac Res 2009;12(3):195-205.

75. Ismail SFH, Johal AS. The role of implants in orthodontics. Journal of Orthodontics 2002;29(3):239-45.

76. Labanauskaite B, Jankauskas G, Vasiliauskas A et al. Implants for orthodontic anchorage. Meta-analysis. Stomatologija 2005;7(4):128-32.

77. Higuchi KW, Slack JM. The use of titanium fixtures for intraoral anchorage to facilitate orthodontic tooth movement. Int J Oral Maxillofac Implants 1991;6(3):338-44.

78. Herrero DB. Implants as anchorage in orthodontics: a clinical case report. J Oral Implantol 1998;24(1):5-10.

79. Kokich VG. Managing complex orthodontic problems: the use of implants for anchorage. Semin Orthod 1996;2(2):153-60.

80. Smalley WM. Implants for tooth movement: determining implant location and orientation. J Esthet Dent 1995;7(2):62-72.

81. Smalley WM, Blanco A. Implants for tooth movement: a fabrication and placement technique for provisional restorations. J Esthet Dent 1995;7(4):150-4.

82. Huang L-H, Shotwell JL, Wang H-L. Dental implants for orthodontic anchorage. American Journal of Orthodontics and Dentofacial Orthopedics 2005;127(6):713-22.

83. Block MS, Hoffman DR. A new device for absolute anchorage for orthodontics. American Journal of Orthodontics and Dentofacial Orthopedics 1995;107(3):251-8.

84. Choi B-H, Zhu S-J, Kim Y-H. A clinical evaluation of titanium miniplates as anchors for orthodontic treatment. American Journal of Orthodontics and Dentofacial Orthopedics 2005;128(3):382-4.

85. Daimaruya T, Nagasaka H, Umemori M et al. The influences of molar intrusion on the inferior alveolar neurovascular bundle and root using the skeletal anchorage system in dogs. The Angle Orthodontist 2001;71(1):60-70.

86. Eroğlu T, Kaya B, Çetinşahin A et al. Success of Zygomatic Plate-Screw Anchorage System. Journal of Oral and Maxillofacial Surgery 2010;68(3):602-5.

87. Sherwood KH, Burch JG, Thompson WJ. Closing anterior open bites by intruding molars with titanium miniplate anchorage. Am J Orthod Dentofacial Orthop 2002;122(6):593-600.

88. Umemori M, Sugawara J, Mitani H et al. Skeletal anchorage system for open-bite correction. American Journal of Orthodontics & Dentofacial Orthopedics 1999;115(2):166-74.

89. Cornelis MA, Scheffler NR, Nyssen-Behets C et al. Patients' and orthodontists' perceptions of miniplates used for temporary skeletal anchorage: A prospective study. American Journal of Orthodontics and Dentofacial Orthopedics 2008;133(1):18-24.

90. Cornelis MA, Scheffler NR, De Clerck HJ et al. Systematic review of the experimental use of temporary skeletal anchorage devices in orthodontics. American Journal of Orthodontics and Dentofacial Orthopedics 2007;131(4):S52-S8.

91. Heymann GC, Cevidanes L, Cornelis M et al. Three-dimensional analysis of maxillary protraction with intermaxillary elastics to miniplates. American Journal of Orthodontics and Dentofacial Orthopedics 2010;137(2):274-84.

92. Furquim B. Hugo De Clerck. 2012.

93. Cevidanes L, Baccetti T, Franchi L et al. Comparison of two protocols for maxillary protraction: bone anchors versus face mask with rapid maxillary expansion. The Angle Orthodontist 2010;80(5):799-806.

94. Crismani AG, Bertl MH, Čelar AG et al. Miniscrews in orthodontic treatment: review and analysis of published clinical trials. American Journal of Orthodontics and Dentofacial Orthopedics 2010;137(1):108-13.

95. Cornelis MA, Scheffler NR, Nyssen-Behets C et al. Patients' and orthodontists' perceptions of miniplates used for temporary skeletal anchorage: A prospective study. American Journal of Orthodontics and Dentofacial Orthopedics 2008;133(1):18-24.

96. Tseng YC, Chen CM, Wang HC et al. Pain perception during miniplate-assisted orthodontic therapy. Kaohsiung J Med Sci 2010;26(11):603-8.

97. Feldmann I, List T, Bondemark L. Orthodontic anchoring techniques and its influence on pain, discomfort, and jaw function--a randomized controlled trial. Eur J Orthod 2012;34(1):102-8.

98. Kuroda S, Sugawara Y, Deguchi T et al. Clinical use of miniscrew implants as orthodontic anchorage: success rates and postoperative discomfort. Am J Orthod Dentofacial Orthop 2007;131(1):9-15.

99. Carano A, Melsen B. Implants in orthodontics. Interview. Prog Orthod 2005;6(1):62-9.

100. Hochman I, Peer G, Grosskopf I et al. Uric acid nephropathy as a presenting sign of chronic lymphocytic leukemia. Clin Nephrol 1995;43(1):64.

101. Kim YH, Yang SM, Kim S et al. Midpalatal miniscrews for orthodontic anchorage: Factors affecting clinical success. American Journal of Orthodontics and Dentofacial Orthopedics 2010;137(1):66-72.

102. Borsos G, Vokó Z, Gredes T et al. Tooth movement using palatal implant supported anchorage compared to conventional dental anchorage. Annals of Anatomy-Anatomischer Anzeiger 2012.

103. Ismail JY. A comparison of current root form implants. Biomechanical design and prosthodontic applications. N Y State Dent J 1989;55(4):34-6.

104. Yamaguchi M, Inami T, Ito K et al. Mini-Implants in the Anchorage Armamentarium: New Paradigms in the Orthodontics. Int J Biomater 2012;2012:8.

105. Kanomi R. Mini-implant for orthodontic anchorage. J Clin Orthod 1997;31(11):763-7.

106. Park HS, Kyung H, Sung J. A simple method of molar uprighting with micro-implant anchorage. Journal of Clinical Orthodontics 2002;36(10):592-6.

107. Lim SM, Hong RK. Distal movement of maxillary molars using a lever-arm and mini-implant system. The Angle Orthodontist 2008;78(1):167-75.

108. Herman RJ, Currier GF, Miyake A. Mini-implant anchorage for maxillary canine retraction: a pilot study. American Journal of Orthodontics and Dentofacial Orthopedics 2006;130(2):228-35.

109. Kim TW, Kim H, Lee SJ. Correction of deep overbite and gummy smile by using a mini-implant with a segmented wire in a growing Class II Division 2 patient. American Journal of Orthodontics and Dentofacial Orthopedics 2006;130(5):676-85.

110. Fritz U, Diedrich P, Kinzinger G et al. The anchorage quality of mini-implants towards translatory and extrusive forces. Journal of Orofacial Orthopedics/Fortschritte der Kieferorthopädie 2003;64(4):293-304.

111. Yao CCJ, Lee JJ, Chen HY et al. Maxillary molar intrusion with fixed appliances and mini-implant anchorage studied in three dimensions. The Angle Orthodontist 2005;75(5):754-60.

112. Kawakami M, Miyawaki S, Noguchi H et al. Screw-type implants used as anchorage for lingual orthodontic mechanics: a case of bimaxillary protrusion with second premolar extraction. The Angle Orthodontist 2004;74(5):715-9.

113. Weyrich C, Noss M, Lisson JA. Comparison of a modified RME appliance with other appliances for transverse maxillary expansion. Journal of Orofacial Orthopedics/Fortschritte der Kieferorthopädie 2010;71(4):265-72.

114. Ge YS, Liu J, Chen L et al. Dentofacial effects of two facemask therapies for maxillary protraction: Miniscrew implants versus rapid maxillary expanders. The Angle Orthodontist 2012;82(6):1083-91.

115. Wilmes B, Nienkemper M, Drescher D. Application and effectiveness of a mini-implant-and tooth-borne rapid palatal expansion device: the hybrid hyrax. World J Orthod 2010;11:323-30.

116. Wilmes B, Drescher D, Nienkemper M. A miniplate system for improved stability of skeletal anchorage. J Clin Orthod 2009;43(8):494-501.

117. Vigolo P, Givani A. Clinical evaluation of single-tooth mini-implant restorations: a five-year retrospective study. J Prosthet Dent 2000;84(1):50-4.

118. Dilek OC, Tezulas E. Treatment of a narrow, single tooth edentulous area with mini-dental implants: a clinical report. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 2007;103(2):e22-e5.

119. Flanagan D. Implant-supported fixed prosthetic treatment using very small-diameter implants: a case report. Journal of Oral Implantology 2006;32(1):34-7.

120. Shatkin TE, Shatkin S, Oppenheimer BD et al. Mini dental implants for long-term fixed and removable prosthetics: a retrospective analysis of 2514 implants placed over a five-year period. Compendium of continuing education in dentistry (Jamesburg, NJ: 1995) 2007;28(2):92.

121. Reynders R, Ronchi L, Bipat S. Mini-implants in orthodontics: A systematic review of the literature. American Journal of Orthodontics and Dentofacial Orthopedics 2009;135(5):564.e1-.e19.

122. Cope JB. Temporary anchorage devices in orthodontics: A paradigm shift. Seminars in orthodontics 2005;11(1):3-9.

123. Lin J, Liou E. A new bone screw for orthodontic anchorage. Journal of clinical orthodontics: JCO 2003;37(12):676.

124. Liou EJW, Pai BCJ, Lin JCY. Do miniscrews remain stationary under orthodontic forces? American Journal of Orthodontics and Dentofacial Orthopedics 2004;126(1):42-7.

125. Melsen B. Mini-implants: where are we? Journal of Clinical Orthodontics 2005;39(9):539.

126. Mah J, Bergstrand F. Temporary anchorage devices: a status report. J Clin Orthod 2005;39(3):132-6.

127. Papadopoulos MA, Tarawneh F. The use of miniscrew implants for temporary skeletal anchorage in orthodontics: a comprehensive review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103(5):21.

128. Christensen GJ. The Increased Use of Small-Diameter Implants. The Journal of the American Dental Association 2009;140(6):709-12.

129. Celenza F, Hochman MN. Absolute anchorage in orthodontics: direct and indirect implant-assisted modalities. Journal of Clinical Orthodontics 2000;34(7):397-402.

130. Ludwig B, Baumgaertel S, Bowman S. Mini-implants in orthodontics. Innovation. Anchorage. Concepts. Quintessence International 2007.

131. Holberg C, Winterhalder P, Holberg N et al. Direct versus indirect loading of orthodontic miniscrew implants—an FEM analysis. Clin Oral Investig 2012:1-7.

132. Heymann GC, Tulloch J. Implantable devices as orthodontic anchorage: a review of current treatment modalities. Journal of Esthetic and Restorative Dentistry 2006;18(2):68-79.

133. Harrison JE. Evidence-based Orthodontics—How do I assess the evidence? Journal of Orthodontics 2000;27(2):189-97.

134. Worthington P, Lang BR, LaVelle WE. Osseointegration in dentistry: an introduction: Quintessence Pub. Co.; 1994.

135. Sherman AJ. Bone reaction to orthodontic forces on vitreous carbon dental implants. Am J Orthod 1978;74(1):79-87.

136. Turley PK, Shapiro PA, Moffett BC. The loading of bioglass-coated aluminium oxide implants to produce sutural expansion of the maxillary complex in the pigtail monkey (Macaca nemestrina). Arch Oral Biol 1980;25(7):459-69.

137. Song HS, Kim YS, Kwon SY et al. A Comparison of the Biocompatibility of Titanium-Base Alloy According to Surface Treatments. Journal of Korean Orthopaedic Research Society 2011;14(2):42-51.

138. Lim YW, Kwon SY, Sun DH et al. The Otto Aufranc Award: Enhanced Biocompatibility of Stainless Steel Implants by Titanium Coating and Microarc Oxidation. Clinical Orthopaedics and Related Research® 2011;469(2):330-8.

139. Steinemann S. Metal implants and surface reactions. Injury 1996;27:S/C16-S/C22.

140. Disegi J, Eschbach L. Stainless steel in bone surgery. Injury 2000;31:D2-D6.

141. Deguchi T, Takano-Yamamoto T, Kanomi R et al. The use of small titanium screws for orthodontic anchorage. Journal of Dental Research 2003;82(5):377-81.

142. Morais LS, Serra GG, Muller CA et al. Titanium alloy mini-implants for orthodontic anchorage: immediate loading and metal ion release. Acta Biomaterialia 2007;3(3):331-9.

143. Carano A, Lonardo P, Velo S et al. Mechanical properties of three different commercially available miniscrews for skeletal anchorage. Prog Orthod 2005;6(1):82-97.

144. Chaddad K, Ferreira AFH, Geurs N et al. Influence of surface characteristics on survival rates of mini-implants. The Angle Orthodontist 2008;78(1):107-13.

145. Galli C, Piemontese M, Ravanetti F et al. Effect of surface treatment on cell responses to grades 4 and 5 titanium for orthodontic mini-implants. American Journal of Orthodontics and Dentofacial Orthopedics 2012;141(6):705-14.

146. Pan C-Y, Chou S-T, Tseng Y-C et al. Influence of different implant materials on the primary stability of orthodontic mini-implants. Kaohsiung J Med Sci 2012;28(12):673-8.

147. Glatzmaier J, Wehrbein H, Diedrich P. Biodegradable implants for orthodontic anchorage. A preliminary biomechanical study. Eur J Orthod 1996;18(5):465-9.

148. Daniel Koo, Cal-Neto JPe, Noronha MdP et al. Alternatives to Skeletal Anchorage in Orthodontics. Orthodontic Cyber Journal 2006;November 2006.

149. Roberts WE, Marshall KJ, Mozsary PG. Rigid endosseous implant utilized as anchorage to protract molars and close an atrophic extraction site. Angle Orthod 1990;60(2):135-52.

150. Turley PK, Kean C, Schur J et al. Orthodontic force application to titanium endosseous implants. Angle Orthod 1988;58(2):151-62.

151. Favero L, Brollo P, Bressan E. Orthodontic anchorage with specific fixtures: Related study analysis. American Journal of Orthodontics and Dentofacial Orthopedics 2002;122(1):84-94.

152. Costa A, Raffainl M, Melsen B. Miniscrews as orthodontic anchorage: a preliminary report. The International journal of adult orthodontics and orthognathic surgery 1998;13(3):201.

153. Jeon M, Kang YG, Mo SS et al. Effects of surface treatment on the osseointegration potential of orthodontic mini-implant. Korean Journal of Orthodontics 2008;38(5):328-36.

154. Kim SH, Lee SJ, Cho IS et al. Rotational resistance of surface-treated mini-implants. The Angle Orthodontist 2009;79(5):899-907.

155. Cope JB. Temporary anchorage devices in orthodontics: a paradigm shift. 2005: p. 3-9.

156. Chung KR, Kim SH, Kook YA. The C-orthodontic micro-implant. Journal of clinical orthodontics: JCO 2004;38(9):478.

157. Vannet BV, Sabzevar MM, Wehrbein H et al. Osseointegration of miniscrews: a histomorphometric evaluation. The European Journal of Orthodontics 2007;29(5):437-42.

158. Favero LG, Pisoni A, Paganelli C. Removal torque of osseointegrated mini-implants: an in vivo evaluation. The European Journal of Orthodontics 2007;29(5):443-8.

159. Freire JN, Silva NR, Gil JN et al. Histomorphologic and histomophometric evaluation of immediately and early loaded mini-implants for orthodontic anchorage. Am J Orthod Dentofacial Orthop 2007;131(6):e1-9.

160. Wilmes B, Ottenstreuer S, Su YY et al. Impact of implant design on primary stability of orthodontic mini-implants. Journal of Orofacial Orthopedics/Fortschritte der Kieferorthopädie 2008;69(1):42-50.

161. Song YY, Cha JY, Hwang CJ. Mechanical characteristics of various orthodontic mini-screws in relation to artificial cortical bone thickness. Angle Orthod 2007;77(6):979-85.

162. Wilmes B, Ottenstreuer S, Su YY et al. Impact of implant design on primary stability of orthodontic mini-implants. J Orofac Orthop 2008;69(1):42-50.

163. Kim JW, Baek SH, Kim TW et al. Comparison of stability between cylindrical and conical type mini-implants. The Angle Orthodontist 2008;78(4):692-8.

164. Holm L, Cunningham SJ, Petrie A et al. An in vitro study of factors affecting the primary stability of orthodontic mini-implants. The Angle Orthodontist 2012.

165. Yoo S-H, Park Y-C, Hwang C-J et al. A comparison of tapered and cylindrical miniscrew stability. The European Journal of Orthodontics 2013:cjt092.

166. Lekholm U, Zarb GA. Patient selection and preparation. Tissue integrated prostheses: osseointegration in clinical dentistry. Chicago: Quintessence Publishing Company;; 1985.

167. Wawrzinek C, Sommer T, Fischer-Brandies H. Microdamage in cortical bone due to the overtightening of orthodontic microscrews. Journal of Orofacial Orthopedics/Fortschritte der Kieferorthopädie 2008;69(2):121-34.

168. Wilmes B, Su YY, Sadigh L et al. Pre-drilling force and insertion torques during orthodontic mini-implant insertion in relation to root contact. Journal of Orofacial Orthopedics/Fortschritte der Kieferorthopädie 2008;69(1):51-8.

169. Motoyoshi M, Hirabayashi M, Uemura M et al. Recommended placement torque when tightening an orthodontic mini-implant. Clin Oral Implants Res 2006;17(1):109-14.

170. Meredith N. Assessment of implant stability as a prognostic determinant. Int J Prosthodont 1998;11(5):491-501.

171. Motoyoshi M, Yano S, Tsuruoka T et al. Biomechanical effect of abutment on stability of orthodontic mini-implant. A finite element analysis. Clin Oral Implants Res 2005;16(4):480-5.

172. Kim YK, Kim YJ, Yun PY et al. Effects of the taper shape, dual-thread, and length on the mechanical properties of mini-implants. The Angle Orthodontist 2009;79(5):908-14.

173. Chen Y, Kyung HM, Gao L et al. Mechanical properties of self-drilling orthodontic micro-implants with different diameters. The Angle Orthodontist 2010;80(5):821-7.

174. Ansell R, Scales JT. A study of some factors which affect the strength of screws and their insertion and holding power in bone. Journal of biomechanics 1968;1(4):279-302.

175. Gantous A, Phillips JH. The effects of varying pilot hole size on the holding power of miniscrews and microscrews. Plast Reconstr Surg 1995;95(7):1165.

176. Fritz U, Ehmer A, Diedrich P. Clinical suitability of titanium microscrews for orthodontic anchorage—preliminary experiences. Journal of Orofacial Orthopedics/Fortschritte der Kieferorthopädie 2004;65(5):410-8.

177. Chen Y, Kyung HM, Zhao WT et al. Critical factors for the success of orthodontic mini-implants: a systematic review. American Journal of Orthodontics and Dentofacial Orthopedics 2009;135(3):284-91.

178. Meursinge Reynders RA, Ronchi L, Ladu L et al. Insertion torque and success of orthodontic mini-implants: A systematic review. American Journal of Orthodontics and Dentofacial Orthopedics 2012;142(5):596-614. e5.

179. Motoyoshi M, Hirabayashi M, Uemura M et al. Recommended placement torque when tightening an orthodontic mini-implant. Clin Oral Implants Res 2005;17(1):109-14.

180. J. Otani HS, K. Ueki, K. Tanne. Aiming for more safety and reliable implantation as an orthodontic anchorage

J Orthod Pract, 3 (2009), pp. 41–50.

181. J.A. Arismendi ZMO, M. Morales, F.J. Gonzalez, P.M. Jaramillo, A. Sanchez. Evaluation of stability of mini implants as bony anchorage for upper molar intrusion

Rev Fac Odontol Univ Antioq, 19 (2007), pp. 59–73.

182. Suzuki EY, Suzuki B. Placement and removal torque values of orthodontic miniscrew implants. American Journal of Orthodontics and Dentofacial Orthopedics 2011;139(5):669-78.

183. Tachibana R, Motoyoshi M, Shinohara A et al. Safe placement techniques for self-drilling orthodontic mini-implants. Int J Oral Maxillofac Surg 2012.

184. Uemura M, Motoyoshi M, Yano S et al. Orthodontic mini-implant stability and the ratio of pilot hole implant diameter. The European Journal of Orthodontics 2012;34(1):52-6.

185. Wilmes B, Panayotidis A, Drescher D. Fracture resistance of orthodontic mini-implants: a biomechanical in vitro study. The European Journal of Orthodontics 2011;33(4):396-401.

186. Büchter A, Wiechmann D, Koerdt S et al. Load-related implant reaction of mini-implants used for orthodontic anchorage. Clin Oral Implants Res 2005;16(4):473-9.

187. Miyawaki S, Koyama I, Inoue M et al. Factors associated with the stability of titanium screws placed in the posterior region for orthodontic anchorage. American Journal of Orthodontics and Dentofacial Orthopedics 2003;124(4):373-8.

188. Costa A, Pasta G, Bergamaschi G. Intraoral hard and soft tissue depths for temporary anchorage devices. 2005: p. 10-5.

189. Wilmes B, Drescher D. Impact of bone quality, implant type, and implantation site preparation on insertion torques of mini-implants used for orthodontic anchorage. Int J Oral Maxillofac Surg 2011;40(7):697-703.

190. Song YY, Cha JY, Hwang CJ. Mechanical characteristics of various orthodontic mini-screws in relation to artificial cortical bone thickness. The Angle Orthodontist 2007;77(6):979-85.

191. Motoyoshi M, Inaba M, Ono A et al. The effect of cortical bone thickness on the stability of orthodontic mini-implants and on the stress distribution in surrounding bone. Int J Oral Maxillofac Surg 2009;38(1):13-8.

192. Baumgaertel S, Hans MG. Buccal cortical bone thickness for mini-implant placement. American Journal of Orthodontics and Dentofacial Orthopedics 2009;136(2):230-5.

193. Laursen MG, Melsen B, Cattaneo PM. An evaluation of insertion sites for mini-implants. The Angle Orthodontist 2012.

194. Jung Y-R, Kim S-C, Kang K-H et al. Placement angle effects on the success rate of orthodontic microimplants and other factors with cone-beam computed tomography. American Journal of Orthodontics and Dentofacial Orthopedics 2013;143(2):173-81.

195. Baumgaertel S. Cortical bone thickness and bone depth of the posterior palatal alveolar process for mini-implant insertion in adults. American Journal of Orthodontics and Dentofacial Orthopedics 2011;140(6):806-11.

196. Ansari T, Mascarenhas R, Husain A. The Relationship of Various Arch Forms and Cortical Bone Thickness. Journal of Dentistry of Tehran University of Medical Sciences 2011;8(1).

197. Park HS, Lee SK, Kwon OW. Group distal movement of teeth using microscrew implant anchorage. The Angle Orthodontist 2005;75(4):602-9.

198. Tseng YC, Hsieh CH, Chen CH et al. The application of mini-implants for orthodontic anchorage. Int J Oral Maxillofac Surg 2006;35(8):704-7.

199. Chen YJ, Chang HH, Huang CY et al. A retrospective analysis of the failure rate of three different orthodontic skeletal anchorage systems. Clin Oral Implants Res 2007;18(6):768-75.

200. Cheng SJ, Tseng IY, Lee JJ et al. A prospective study of the risk factors associated with failure of mini-implants used for orthodontic anchorage. International Journal of Oral and Maxillofacial Implants 2004;19(1):100-6.

201. Kanomi R. Mini-implant for orthodontic anchorage. Journal of clinical orthodontics: JCO 1997;31(11):763.

202. Chen CH, Chang CS, Hsieh CH et al. The use of microimplants in orthodontic anchorage. Journal of Oral and Maxillofacial Surgery 2006;64(8):1209-13.

203. Sarul M, Minch L, Park H-S et al. Effect of the length of orthodontic mini-screw implants on their long-term stability: A prospective study. Angle Orthodontist 2014.

204. Kuroda S, Sugawara Y, Deguchi T et al. Clinical use of miniscrew implants as orthodontic anchorage: success rates and postoperative discomfort. American Journal of Orthodontics and Dentofacial Orthopedics 2007;131(1):9-15.

205. Lin T-S, Tsai F-D, Chen C-Y et al. Factorial analysis of variables affecting bone stress adjacent to the orthodontic anchorage mini-implant with finite element analysis. American Journal of Orthodontics and Dentofacial Orthopedics 2013;143(2):182-9.

206. Renouard F, Nisand D. Impact of implant length and diameter on survival rates. Clin Oral Implants Res 2006;17(S2):35-51.

207. Freudenthaler JW, Bantleon HP, Haas R. Bicortical titanium screws for critical orthodontic anchorage in the mandible: a preliminary report on clinical applications. Clin Oral Implants Res 2001;12(4):358-63.

208. Ivanoff CJ, Grondahl K, Bergstrom C et al. Influence of bicortical or monocortical anchorage on maxillary implant stability: a 15-year retrospective study of Branemark System implants. Int J Oral Maxillofac Implants 2000;15(1):103-10.

209. Morarend C, Qian F, Marshall SD et al. Effect of screw diameter on orthodontic skeletal anchorage. American Journal of Orthodontics and Dentofacial Orthopedics 2009;136(2):224-9.

210. Park HS, Jeong SH, Kwon OW. Factors affecting the clinical success of screw implants used as orthodontic anchorage. American Journal of Orthodontics and Dentofacial Orthopedics 2006;130(1):18-25.

211. Berens A, Wiechmann D, Dempf R. Mini-and micro-screws for temporary skeletal anchorage in orthodontic therapy. Journal of Orofacial Orthopedics/Fortschritte der Kieferorthopädie 2006;67(6):450-8.

212. Ohmae M, Saito S, Morohashi T et al. A clinical and histological evaluation of titanium mini-implants as anchors for orthodontic intrusion in the beagle dog. Am J Orthod Dentofacial Orthop 2001;119(5):489-97.

213. Park HS. Clinical study on success rate of microscrew implants for orthodontic anchorage. Korean J Orthod 2003;33(3):151-6.

214. Poggio PM, Incorvati C, Velo S et al. “Safe Zones”: A Guide for Miniscrew Positioning in the Maxillary and Mandibular Arch. The Angle Orthodontist 2006;76(2):191-7.

215. Asscherickx K, Vannet BV, Wehrbein H et al. Root repair after injury from mini-screw. Clin Oral Implants Res 2005;16(5):575-8.

216. Chen YH, Chang HH, Chen YJ et al. Root contact during insertion of miniscrews for orthodontic anchorage increases the failure rate: an animal study. Clin Oral Implants Res 2008;19(1):99-106.

217. Ahmed V KS, Rooban T, Krishnaswamy NR et al. Root damage and repair in patients with temporary skeletal anchorage devices. American Journal of Orthodontics and Dentofacial Orthopedics 2012;141(5):547-55.

218. Downs WB. The role of cephalometrics in orthodontic case analysis and diagnosis. American Journal of Orthodontics 1952;38(3):162-82.

219. Truhlar RS, Morris HF, Ochi S. A review of panoramic radiography and its potential use in implant dentistry. Implant Dentistry 1993;2(2):122-30.

220. Duckworth J, Judy P, Goodson J et al. A Method for the Geometric and Densitometric Standardization of Intraoral Radiographs*. J Periodontol 1983;54(7):435-40.

221. Chen YJ, Chang HH, Lin HY et al. Stability of miniplates and miniscrews used for orthodontic anchorage: experience with 492 temporary anchorage devices. Clin Oral Implants Res 2008;19(11):1188-96.

222. Lou L, Lagravere MO, Compton S et al. Accuracy of measurements and reliability of landmark identification with computed tomography (CT) techniques in the maxillofacial area: a systematic review. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 2007;104(3):402-11.

223. Kim KD, Ruprecht A, Wang G et al. Accuracy of facial soft tissue thickness measurements in personal computer-based multiplanar reconstructed computed tomographic images. Forensic science international 2005;155(1):28-34.

224. Cavalcanti MGP, Ruprecht A, Bonomie JM et al. The validation of 3D spiral CT-based measurements of simulated maxillofacial neoplasms. Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics 2000;89:753-8.

225. Cavalcanti MGP, Ruprecht A, Bonomie JM et al. Accuracy and precision of spiral CT in the assessment of neoplastic lesions associated with the mandible. Acad Radiol 2000;7(2):97-9.

226. Pinsky H, Dyda S, Pinsky R et al. Accuracy of three-dimensional measurements using cone-beam CT. Dentomaxillofacial Radiology 2006;35(6):410-6.

227. Kobayashi K, Shimoda S, Nakagawa Y et al. Accuracy in measurement of distance using limited cone-beam computerized tomography. Int J Oral Maxillofac Implants 2004;19(2):228.

228. Suomalainen A, Vehmas T, Kortesniemi M et al. Accuracy of linear measurements using dental cone beam and conventional multislice computed tomography. Dentomaxillofacial Radiology 2008;37(1):10-7.

229. Loubele M, Bogaerts R, Van Dijck E et al. Comparison between effective radiation dose of CBCT and MSCT scanners for dentomaxillofacial applications. Eur J Radiol 2009;71(3):461-8.

230. Liu H, Liu D, Wang G et al. Accuracy of surgical positioning of orthodontic miniscrews with a computer-aided design and manufacturing template. American Journal of Orthodontics and Dentofacial Orthopedics 2010;137(6):728. e1-. e10.

231. Yu JJ, Kim GT, Choi YS et al. Accuracy of a cone beam computed tomography-guided surgical stent for orthodontic mini-implant placement. The Angle Orthodontist 2011;82(2):275-83.

232. Qiu L, Haruyama N, Suzuki S et al. Accuracy of orthodontic miniscrew implantation guided by stereolithographic surgical stent based on cone-beam CT-derived 3D images. The Angle Orthodontist 2011;82(2):284-93.

233. Jung BA, Wehrbein H, Wagner W et al. Preoperative Diagnostic for Palatal Implants: Is CT or CBCT Necessary? Clin Implant Dent Relat Res 2012;14(3):400-5.

234. Eriksson A, Albrektsson T. Temperature threshold levels for heat-induced bone tissue injury: a vital-microscopic study in the rabbit. J Prosthet Dent 1983;50(1):101.

235. Eriksson R, Albrektsson T. The effect of heat on bone regeneration: an experimental study in the rabbit using the bone growth chamber. Journal of Oral and Maxillofacial Surgery 1984;42(11):705-11.

236. Wilmes B, Su YY, Drescher D. Insertion angle impact on primary stability of orthodontic mini-implants. The Angle Orthodontist 2008;78(6):1065-70.

237. Wilmes B, Drescher D. Impact of insertion depth and predrilling diameter on primary stability of orthodontic mini-implants. The Angle Orthodontist 2009;79(4):609-14.

238. Warrer K, Buser D, Lang N et al. Plaque-induced peri-implantitis in the presence or absence of keratinized mucosa. An experimental study in monkeys. Clin Oral Implants Res 2002;6(3):131-8.

239. Maino BG, Mura P, Bednar J. Miniscrew implants: the spider screw anchorage system. 2005: p. 40-6.

240. Chopra SS, Chakranarayan A. Clinical evaluation of immediate loading of titanium orthodontic implants. Medical Journal Armed Forces India (0).

241. Zhao L, Xu Z, Yang Z et al. Orthodontic mini-implant stability in different healing times before loading: A microscopic computerized tomographic and biomechanical analysis. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 2009;108(2):196-202.

242. Yano S, Motoyoshi M, Inaba M et al. A healing period increases mini-implant stability in growing rats. J Oral Sci 2014;56(2):113-8.

243. Nienkemper M, Wilmes B, Pauls A et al. Mini-implant stability at the initial healing period: A clinical pilot study. The Angle Orthodontist 2013;84(1):127-33.

244. Pittman JW, Navalgund A, Byun SH et al. Primary migration of a mini-implant under a functional orthodontic loading. Clin Oral Investig 2014;18(3):721-8.

245. Nienkemper M, Handschel J, Drescher D. Systematic review of mini-implant displacement under orthodontic loading. International journal of oral science 2014;6(1):1-6.

246. Bain CA. Smoking and implant failure--benefits of a smoking cessation protocol. Int J Oral Maxillofac Implants 1996;11(6):756.

247. Bain C, Moy P. The association between the failure of dental implants and cigarette smoking. Int J Oral Maxillofac Implants 1993;8(6):609.

248. Kuitert RBR. [Smoking and orthodontics]. Ned Tijdschr Tandheelkd 2006;113(12):506-12.

249. Wennström JL, Stokland BL, Nyman S et al. Periodontal tissue response to orthodontic movement of teeth with infrabony pockets. American Journal of Orthodontics and Dentofacial Orthopedics 1993;103(4):313-9.

250. Burden D, Mullally B, Sandler J. Orthodontic treatment of patients with medical disorders. The European Journal of Orthodontics 2001;23(4):363-72.

251. Baumgaertel S. Hard and soft tissue considerations at mini-implant insertion sites. Journal of Orthodontics 2014;41:s3.

252. Kuroda S, Yamada K, Deguchi T et al. Root proximity is a major factor for screw failure in orthodontic anchorage. American Journal of Orthodontics and Dentofacial Orthopedics 2007;131(4):S68-S73.

253. Motoyoshi M, Uchida Y, Matsuoka M et al. Assessment of damping capacity as an index of root proximity in self-drilling orthodontic mini-implants. Clin Oral Investig 2014;18(1):321-6.

254. AlSamak S, Gkantidis N, Bitsanis E et al. Assessment of Potential Orthodontic Mini-implant Insertion Sites Based on Anatomical Hard Tissue Parameters: A Systematic Review. Int J Oral Maxillofac Implants 2012;27(4):875.

255. Cope JB. Temporary anchorage devices in orthodontics: a paradigm shift. In: Seminars in orthodontics, 2005: p. 3-9.

256. Baumgaertel S. Quantitative investigation of palatal bone depth and cortical bone thickness for mini-implant placement in adults. American Journal of Orthodontics and Dentofacial Orthopedics 2009;136(1):104-8.

257. Nienkemper M, Pauls A, Ludwig B et al. Multifunctional Use of Palatal Mini-Implants. Journal of Clinical Orthodontics 2012;46(11):679.

258. Papageorgiou SN, Zogakis IP, Papadopoulos MA. Failure rates and associated risk factors of orthodontic miniscrew implants: A meta-analysis. American Journal of Orthodontics and Dentofacial Orthopedics 2012;142(5):577-95.e7.

259. Berens A, Wiechmann D, Rudiger J. Skeletal anchorage in orthodontics with mini-and microscrews. International Orthodontics 2005;3(3):235.

260. Wiechmann D, Meyer U, Büchter A. Success rate of mini-and micro-implants used for orthodontic anchorage: a prospective clinical study. Clin Oral Implants Res 2007;18(2):263-7.

261. Koldsland OC, Scheie AA, Aass AM. Prevalence of peri-implantitis related to severity of the disease with different degrees of bone loss. J Periodontol 2010;81(2):231-8.

262. Renvert S, Aghazadeh A, Hallström H et al. Factors related to peri-implantitis–a retrospective study. Clin Oral Implants Res 2014;25(4):522-9.

263. Romeo E, Ghisolfi M, Carmagnola D. Peri-implant diseases. A systematic review of the literature. Minerva Stomatol 2004;53(5):215-30.

264. Kravitz ND, Kusnoto B. Risks and complications of orthodontic miniscrews. American Journal of Orthodontics and Dentofacial Orthopedics 2007;131(4):S43-S51.

265. Park H-S, Jeong S-H, Kwon O-W. Factors affecting the clinical success of screw implants used as orthodontic anchorage. American Journal of Orthodontics and Dentofacial Orthopedics 2006;130(1):18-25.

266. Rabe N. variations Root injuries caused by orthodontic mini-implants; 2013.

267. Farinazzo Vitral R, Santiago R, Oliveira G et al. Mini-Implants: When Orthodontists are Caught in their Own Web. J Clin Case Rep 2012;2(130):2.

268. Agrawal N, Kallury A, Agrawal K et al. Alveolar bone exostoses subsequent to orthodontic implant placement. BMJ Case Rep 2013;25(10):2012-007951.

269. Livas C, Delli K, Karapsias S et al. Investigation of bacteremia induced by removal of orthodontic mini-implants. The European Journal of Orthodontics 2013.

270. Wilmes B, Rademacher C, Olthoff G et al. Parameters affecting primary stability of orthodontic mini-implants. Journal of Orofacial Orthopedics/Fortschritte der Kieferorthopädie 2006;67(3):162-74.

271. Melsen B, Costa A. Immediate loading of implants used for orthodontic anchorage. Clin Orthod Res 2000;3(1):23-8.

272. Dalessandri D, Salgarello S, Dalessandri M et al. Determinants for success rates of temporary anchorage devices in orthodontics: a meta-analysis (n> 50). Eur J Orthod 2013;36:303-13.

273. Kohavi D, Klinger A, Steinberg D et al. Adsorption of salivary proteins onto prosthetic titanium components. J Prosthet Dent 1995;74(5):531-4.

274. Fürst MM, Salvi GE, Lang NP et al. Bacterial colonization immediately after installation on oral titanium implants. Clin Oral Implants Res 2007;18(4):501-8.

275. Lee A, Wang H-L. Biofilm related to dental implants. Implant Dentistry 2010;19(5):387-93.

276. Costerton J, Montanaro L, Arciola C. Biofilm in implant infections: its production and regulation. The International journal of artificial organs 2005;28(11):1062-8.

277. Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. Periodontology 2000 2002;28(1):12-55.

278. Mombelli A, Oosten M, Schürch E et al. The microbiota associated with successful or failing osseointegrated titanium implants. Oral microbiology and immunology 1987;2(4):145-51.

279. Quirynen M, Vogels R, Pauwels M et al. Initial subgingival colonization of 'pristine'pockets. Journal of Dental Research 2005;84(4):340-4.

280. Lee K, Maiden M, Tanner A et al. Microbiota of successful osseointegrated dental implants. J Periodontol 1999;70(2):131-8.

281. Leonhardt Å, Renvert S, Dahlén G. Microbial findings at failing implants. Clin Oral Implants Res 1999;10(5):339-45.

282. Schierano G, Pejrone G, Roana J et al. A split-mouth study on microbiological profile in clinical healthy teeth and implants related to key inflammatory mediators. International journal of immunopathology and pharmacology 2009;23(1):279-88.

283. Ericsson I, Berglundh T, Marinello C et al. Long-standing plaque and gingivitis at implants and teeth in the dog. Clin Oral Implants Res 1992;3(3):99-103.

284. Chin MYH, Sandham A, de Vries J et al. Biofilm formation on surface characterized micro-implants for skeletal anchorage in orthodontics. Biomaterials 2007;28(11):2032-40.

285. Buser D, Schenk R, Steinemann S et al. Influence of surface characteristics on bone integration of titanium implants. A histomorphometric study in miniature pigs. Journal of biomedical materials research 1991;25(7):889-902.

286. Shalabi M, Gortemaker A, Van't Hof M et al. Implant surface roughness and bone healing: a systematic review. Journal of Dental Research 2006;85(6):496-500.

287. Lang NP, Jepsen S. Implant surfaces and design (Working Group 4). Clin Oral Implants Res 2009;20(s4):228-31.

288. Teughels W, Van Assche N, Sliepen I et al. Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res 2006;17(S2):68-81.

289. Baldi D, Menini M, Pera F et al. Plaque accumulation on exposed titanium surfaces and peri-implant tissue behavior. A preliminary 1-year clinical study. Int J Prosthodont 2008;22(5):447-55.

290. Quirynen M, Abarca M, Van Assche N et al. Impact of supportive periodontal therapy and implant surface roughness on implant outcome in patients with a history of periodontitis. Journal of clinical periodontology 2007;34(9):805-15.

291. Albouy JP, Abrahamsson I, Persson LG et al. Spontaneous progression of ligatured induced peri-implantitis at implants with different surface characteristics. An experimental study in dogs II: histological observations. Clin Oral Implants Res 2009;20(4):366-71.

292. Berglundh T, Gotfredsen K, Zitzmann N et al. Spontaneous progression of ligature induced peri-implantitis at implants with different surface roughness: an experimental study in dogs. Clin Oral Implants Res 2007;18(5):655-61.

293. Amarante E, Chambrone L, Lotufo R et al. Early dental plaque formation on toothbrushed titanium implant surfaces. American journal of dentistry 2008;21(5):318-22.

294. Elter C, Heuer W, Demling A et al. Supra-and subgingival biofilm formation on implant abutments with different surface characteristics. Int J Oral Maxillofac Implants 2007;23(2):327-34.

295. Quirynen M, Van der Mei H, Bollen C et al. An in vivo study of the influence of the surface roughness of implants on the microbiology of

supra-and subgingival plaque. Journal of Dental Research 1993;72(9):1304-9.

296. Wilson M, Pratten J. Laboratory assessment of antimicrobials for plaque-related diseases. 1999.

297. Auschill TM, Hein N, Hellwig E et al. Effect of two antimicrobial agents on early in situ biofilm formation. Journal of clinical periodontology 2005;32(2):147-52.

298. Dodds MW, Johnson DA, Yeh C-K. Health benefits of saliva: a review. Journal of dentistry 2005;33(3):223-33.

299. Mathur S, Mathur T, Srivastava R et al. Chlorhexidine: the gold standard in chemical plaque control. Natl J Physiol Pharm Pharmacol 2011;1:45-50.

300. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clinical microbiology reviews 1999;12(1):147-79.

301. Mohammadi Z, Abbott P. The properties and applications of chlorhexidine in endodontics. International Endodontic Journal 2009;42(4):288-302.

302. García-Caballero L, Quintas V, Prada-López I et al. Chlorhexidine Substantivity on Salivary Flora and Plaque-Like Biofilm: An In Situ Model. PLoS One 2013;8(12):e83522.

303. Li X, Wang J, Joiner A et al. The remineralisation of enamel: a review of the literature. Journal of dentistry 2014;42:S12-S20.

304. Cochrane N, Cai F, Huq N et al. New approaches to enhanced remineralization of tooth enamel. Journal of Dental Research 2010;89(11):1187-97.

305. Breaker R. New insight on the response of bacteria to fluoride. Caries research 2012;46(1):78-81.

306. Yoshinari M, Oda Y, Kato T et al. Influence of surface modifications to titanium on antibacterial activity in vitro. Biomaterials 2001;22(14):2043-8.

307. Wu H-J, Huang L-L, Chen S-W et al. Surface characterization of anodized dental archwires and miniscrews. Journal of the Taiwan Institute of Chemical Engineers 2009;40(5):563-72.

308. Larsson C, Thomsen P, Aronsson B-O et al. Bone response to surface-modified titanium implants: studies on the early tissue response to machined and electropolished implants with different oxide thicknesses. Biomaterials 1996;17(6):605-16.

309. Boyan BD, Hummert TW, Dean DD et al. Role of material surfaces in regulating bone and cartilage cell response. Biomaterials 1996;17(2):137-46.

310. Kotsovilis S, Karoussis IK, Trianti M et al. Therapy of peri-implantitis: a systematic review. Journal of clinical periodontology 2008;35(7):621-9.

311. Heydenrijk K, Meijer H, van der Reijden WA et al. Microbiota around root-form endosseous implants: a review of the literature. Int J Oral Maxillofac Implants 2001;17(6):829-38.

312. Shalabi MM, Gortemaker A, Hof MAVt et al. Implant Surface Roughness and Bone Healing: a Systematic Review. Journal of Dental Research 2006;85(6):496-500.

313. Choi S-H, Cha J-Y, Joo U-H et al. Surface changes of anodic oxidized orthodontic titanium miniscrew. The Angle Orthodontist 2011;82(3):522-8.

314. Fröjd V, Chávez de Paz L, Andersson M et al. In situ analysis of multispecies biofilm formation on customized titanium surfaces. Molecular oral microbiology 2011;26(4):241-52.

315. Schmidlin PR, MULLER P, Attin T et al. Polyspecies biofilm formation on implant surfaces with different surface characteristics. Journal of Applied Oral Science 2013;21(1):48-55.

316. He Y. VISCOELASTICITY OF ORAL BIOFILMS AND ANTIMICROBIAL PENETRATION. 2013.

317. Helmerhorst EJ, Hodgson R, Van't Hof W et al. The effects of histatin-derived basic antimicrobial peptides on oral biofilms. Journal of Dental Research 1999;78(6):1245-50.

318. Pratten J, Smith A, Wilson M. Response of single species biofilms and microcosm dental plaques to pulsing with chlorhexidine. Journal of Antimicrobial Chemotherapy 1998;42(4):453-9.

319. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent 2001;85(2):162-9.

320. Tenovuo J. Antimicrobial function of human saliva-how important is it for oral health? Acta Odontologica 1998;56(5):250-6.

321. Revie RW, Greene ND. Corrosion behaviour of surgical implant materials: I. Effects of sterilization. Corrosion Science 1969;9(10):755-62.

322. AlSamak S, Bitsanis E, Makou M et al. Morphological and structural characteristics of orthodontic mini-implants. Journal of Orofacial Orthopedics/Fortschritte der Kieferorthopädie 2012;73(1):58-71.

323. Wennerberg A, Albrektsson T. Effects of titanium surface topography on bone integration: a systematic review. Clin Oral Implants Res 2009;20(s4):172-84.

324. Albrektsson T, Wennerberg A. Oral implant surfaces: Part 1--review focusing on topographic and chemical properties of different surfaces and in vivo responses to them. Int J Prosthodont 2003;17(5):536-43.

325. Bartold P, Kuliwaba JS, Lee V et al. Influence of surface roughness and shape on microdamage of the osseous surface adjacent to titanium dental implants. Clin Oral Implants Res 2011;22(6):613-8.

326. Palmquist A, Engqvist H, Lausmaa J et al. Commercially available dental implants: review of their surface characteristics. Journal of Biomaterials and Tissue Engineering 2012;2(2):112-24.

327. Lin HY, Liu Y, Wismeijer D et al. Effects of oral implant surface roughness on bacterial biofilm formation and treatment efficacy. Int J Oral Maxillofac Implants 2012;28(5):1226-31.

328. Hahnel S, Wieser A, Lang R et al. Biofilm formation on the surface of modern implant abutment materials. Clin Oral Implants Res 2014.

329. Subramani K, Jung RE, Molenberg A et al. Biofilm on dental implants: a review of the literature. Int J Oral Maxillofac Implants 2008;24(4):616-26.

330. da Silva CHFP, Vidigal Jr GM, de Uzeda M et al. Influence of titanium surface roughness on attachment of Streptococcus sanguis: an in vitro study. Implant Dentistry 2005;14(1):88-93.

331. Bürgers R, Gerlach T, Hahnel S et al. In vivo and in vitro biofilm formation on two different titanium implant surfaces. Clin Oral Implants Res 2010;21(2):156-64.

332. Almaguer-Flores A, Ximénez-Fyvie L, Rodil S. Oral bacterial adhesion on amorphous carbon and titanium films: effect of surface roughness and culture media. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2010;92(1):196-204.

333. Mei L, Busscher HJ, van der Mei HC et al. Influence of surface roughness on streptococcal adhesion forces to composite resins. Dental Materials 2011;27(8):770-8.

334. Park J, Song C, Jung J et al. The effects of surface roughness of composite resin on biofilm formation of Streptococcus mutans in the presence of saliva. Oper Dent 2012;37(5):532-9.

335. Berger JC, Driscoll CF, Romberg E et al. Surface roughness of denture base acrylic resins after processing and after polishing. Journal of Prosthodontics 2006;15(3):180-6.

336. Verran J, Maryan CJ. Retention of Candida albicans on acrylic resin and silicone of different surface topography. J Prosthet Dent 1997;77(5):535-9.

337. Kim J-D, Pyun S-I, Seo M. Effect of hydrogen on stresses in anodic oxide film on titanium. Electrochimica acta 2003;48(9):1123-30.

338. Dunn D, Raghavan S. Formation and characterization of anodized layers on CP Ti and Ti-6Al-4V biomaterials. Surface and Coatings Technology 1992;50(3):223-32.

339. Duarte LT, Bolfarini C, Biaggio SR et al. Growth of aluminum-free porous oxide layers on titanium and its alloys Ti-6Al-4V and Ti-6Al-7Nb by micro-arc oxidation. Materials Science and Engineering: C 2014;41:343-8.

340. Javed F, Al-Hezaimi K, Almas K et al. Is titanium sensitivity associated with allergic reactions in patients with dental implants? A systematic review. Clin Implant Dent Relat Res 2013;15(1):47-52.

341. Manivasagam G, Dhinasekaran D, Rajamanickam A. Biomedical implants: Corrosion and its prevention-a review. Recent Patents on Corrosion Science 2010;2(1):40-54.

342. Omar O, Svensson S, Zoric N et al. In vivo gene expression in response to anodically oxidized versus machined titanium implants. Journal of Biomedical Materials Research Part A 2010;92(4):1552-66.

343. Karmarker S, Yu W, Kyung H-M. Effect of surface anodization on stability of orthodontic microimplant. Korean Journal of Orthodontics 2012;42(1):4-10.

344. Sul Y-T, Johansson CB, Jeong Y et al. The electrochemical oxide growth behaviour on titanium in acid and alkaline electrolytes. Med Eng Phys 2001;23(5):329-46.

345. Narayanan R, Seshadri S. Phosphoric acid anodization of Ti–6Al–4V–Structural and corrosion aspects. Corrosion Science 2007;49(2):542-58.

346. Kim K-H, Ramaswamy N. Electrochemical surface modification of titanium in dentistry. Dent Mater J 2009;28(1):20-36.

347. Ferdjani S, David D, Beranger G. Anodic oxidation of titanium in phosphoric acid baths: Phosphorus incorporation into the oxide. Journal of alloys and compounds 1993;200(1):191-4.

348. Cigada A, Cabrini M, Pedeferri P. Increasing of the corrosion resistance of the Ti6Al4V alloy by high thickness anodic oxidation. Journal of Materials Science: Materials in Medicine 1992;3(6):408-12.

349. Simka W. Preliminary investigations on the anodic oxidation of Ti–13Nb–13Zr alloy in a solution containing calcium and phosphorus. Electrochimica acta 2011;56(27):9831-7.

350. Zhu X, Kim K-H, Jeong Y. Anodic oxide films containing Ca and P of titanium biomaterial. Biomaterials 2001;22(16):2199-206.

351. Serro A, Saramago B. Influence of sterilization on the mineralization of titanium implants induced by incubation in various biological model fluids. Biomaterials 2003;24(26):4749-60.

352. Ehrenfest DMD, Coelho PG, Kang B-S et al. Classification of osseointegrated implant surfaces: materials, chemistry and topography. Trends in biotechnology 2010;28(4):198-206.

353. Del Curto B, Brunella M, Giordano C et al. Decreased bacterial adhesion to surface-treated titanium. International journal of artificial organs 2005;28(7):718-30.

354. Zhao L, Chu PK, Zhang Y et al. Antibacterial coatings on titanium implants. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2009;91(1):470-80.

355. Puckett SD, Taylor E, Raimondo T et al. The relationship between the nanostructure of titanium surfaces and bacterial attachment. Biomaterials 2010;31(4):706-13.

356. Tanner A, Maiden M, Lee K et al. Dental implant infections. Clinical Infectious Diseases 1997;25(Supplement 2):S213-S7.

357. Kolenbrander PE, Palmer RJ, Periasamy S et al. Oral multispecies biofilm development and the key role of cell–cell distance. Nature Reviews Microbiology 2010;8(7):471-80.

358. Roos-Jansåker AM, Renvert S, Egelberg J. Treatment of peri-implant infections: a literature review. Journal of clinical periodontology 2003;30(6):467-85.

359. Marsh P. Are dental diseases examples of ecological catastrophes? Microbiology 2003;149(2):279-94.

360. Marsh P. Dental plaque: biological significance of a biofilm and community life-style. Journal of clinical periodontology 2005;32(s6):7-15.

361. Marsh P. Controlling the oral biofilm with antimicrobials. Journal of dentistry 2010;38:S11-S5.

362. Park KD, Kim YS, Han DK et al. Bacterial adhesion on PEG modified polyurethane surfaces. Biomaterials 1998;19(7-9):851-9.

363. Busscher HJ, Weerkamp AH. Specific and non-specific interactions in bacterial adhesion to solid substrata. FEMS Microbiology Reviews 1987;3(2):165-73.

364. Hermansson M. The DLVO theory in microbial adhesion. Colloids and Surfaces B: Biointerfaces 1999;14(1):105-19.

365. Gottenbos B, Grijpma DW, van der Mei HC et al. Antimicrobial effects of positively charged surfaces on adhering Gram-positive and Gram-negative bacteria. Journal of Antimicrobial Chemotherapy 2001;48(1):7-13.

366. Kang C-K, Lee Y-S. The surface modification of stainless steel and the correlation between the surface properties and protein adsorption. Journal of Materials Science: Materials in Medicine 2007;18(7):1389-98.

367. Pedersen K. Biofilm development on stainless steel and PVC surfaces in drinking water. Water Research 1990;24(2):239-43.

368. Baier R, Meyer A, Natiella J et al. Surface properties determine bioadhesive outcomes: methods and results. Journal of biomedical materials research 1984;18(4):337-55.

369. Tengvall P. Proteins at titanium interfaces. In: Titanium in Medicine: Springer; 2001. p. 457-83.

370. Bolanča Z, Hladnik A. Some properties of the anodized aluminium surface. 15th WCNDT Roma 2000 2000.

371. Maddikeri R, Tosatti S, Schuler M et al. Reduced medical infection related bacterial strains adhesion on bioactive RGD modified titanium surfaces: a first step toward cell selective surfaces. Journal of Biomedical Materials Research Part A 2008;84(2):425-35.

372. Busscher H, Rinastiti M, Siswomihardjo W et al. Biofilm formation on dental restorative and implant materials. Journal of Dental Research 2010;89(7):657-65.

373. Harris L, Tosatti S, Wieland M et al. Staphylococcus aureus adhesion to titanium oxide surfaces coated with non-functionalized and peptide-functionalized poly (L-lysine)-grafted-poly (ethylene glycol) copolymers. Biomaterials 2004;25(18):4135-48.

374. Van der Mei H, White D, Atema-Smit J et al. A method to study sustained antimicrobial activity of rinse and dentifrice components on biofilm viability in vivo. Journal of clinical periodontology 2006;33(1):14-20.

375. Netuschil L, Weiger R, Preisler R et al. Plaque bacteria counts and vitality during chlorhexidine, meridol and listerine mouthrinses. European Journal of Oral Sciences 1995;103(6):355-61.

376. Kozlovsky A, Artzi Z, Moses O et al. Interaction of chlorhexidine with smooth and rough types of titanium surfaces. J Periodontol 2006;77(7):1194-200.

377. Ryu H. Chlorhexidine uptake and release from modified titanium surfaces. 2014.

378. Barbour ME, Gandhi N, el-Turki A et al. Differential adhesion of streptococcus gordonii to anatase and rutile titanium dioxide surfaces with and without functionalization with chlorhexidine. Journal of Biomedical Materials Research Part A 2009;90(4):993-8.

379. Schiff N, Grosgogeat B, Lissac M et al. Influence of fluoride content and pH on the corrosion resistance of titanium and its alloys. Biomaterials 2002;23(9):1995-2002.

380. Reclaru L, Meyer J-M. Effects of fluorides on titanium and other dental alloys in dentistry. Biomaterials 1998;19(1-3):85-92.

381. Toumelin-Chemla F, Rouelle F, Burdairon G. Corrosive properties of fluoride-containing odontologic gels against titanium. Journal of dentistry 1996;24(1):109-15.

382. Noguti J, de Oliveira F, Peres RC et al. The role of fluoride on the process of titanium corrosion in oral cavity. BioMetals 2012;25(5):859-62.

383. Walker MP, White RJ, Kula KS. Effect of fluoride prophylactic agents on the mechanical properties of nickel-titanium-based orthodontic wires. American Journal of Orthodontics and Dentofacial Orthopedics 2005;127(6):662-9.

384. Eliades T, Athanasiou AE. In vivo aging of orthodontic alloys: implications for corrosion potential, nickel release, and biocompatibility. The Angle Orthodontist 2002;72(3):222-37.

385. Kao C-T, Ding S-J, He H et al. Cytotoxicity of orthodontic wire corroded in fluoride solution in vitro. The Angle Orthodontist 2007;77(2):349-54.

386. Adya N, Alam M, Ravindranath T et al. Corrosion in titanium dental implants: literature review. The Journal 2005;5(3):127.

387. Twetman S, Axelsson S, Dahlgren H et al. Caries-preventive effect of fluoride toothpaste: a systematic review. Acta Odontologica 2003;61(6):347-55.

388. Kay E, Locker D. A systematic review of the effectiveness of health promotion aimed at improving oral health. Community Dental Health 1998;15(3):132-44.

389. McDonagh MS, Whiting PF, Wilson PM et al. Systematic review of water fluoridation. Bmj 2000;321(7265):855-9.