The Bone Anchored Hearing System:

Understanding and improving clinical outcomes

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door

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Table of Contents

Chapter 1

Introduction & thesis outline

Introduction

Air conduction hearing

Anatomy

Hearing is the result of a series of complex mechanisms. In the normal situation, air conduction hearing is considered the main pathway for hearing. The human ear consists out of an outer ear, a middle ear and an inner ear (Figure 1). The outer ear is composed of the pinna and external auditory canal. The middle ear contains the tympanic membrane (ear drum) and the tympanic space in which three ossicles are located from lateral to medial: malleus, incus and stapes. The eardrum is connected to the malleus which in turn is connected to the incus. The incus is connected to the stapes that attaches to the oval window of the inner ear. The inner ear includes the cochlea and the organ of equilibrium. The cochlea is a shell shaped organ in which three distinct compartments can be distinguished: the scala vestibuli, scala media and scala tympani. The oval window is attached to the scala vestibuli. The scala tympani ends in the round window.

Function

The pinna itself has a distinct shape which helps to localize sounds. Vibrating air molecules elicited by a sound source enter the auditory canal and stimulate the ear drum. The shape of the external auditory canal can amplify sound between 3000-4000 Hertz. The area of the eardrum is approximately 20 times larger than the oval window. The malleus-incus complex has a lever function. Both attribute to signal enhancement in the middle-ear. The oval and round windows allow for fluid movement in the scala vestibuli and scala tympani. Fluid movements stimulate hair cells which are transferred to nerve excitations in the auditory nerve. The excitations are processed in the brain resulting in the perception of sound.

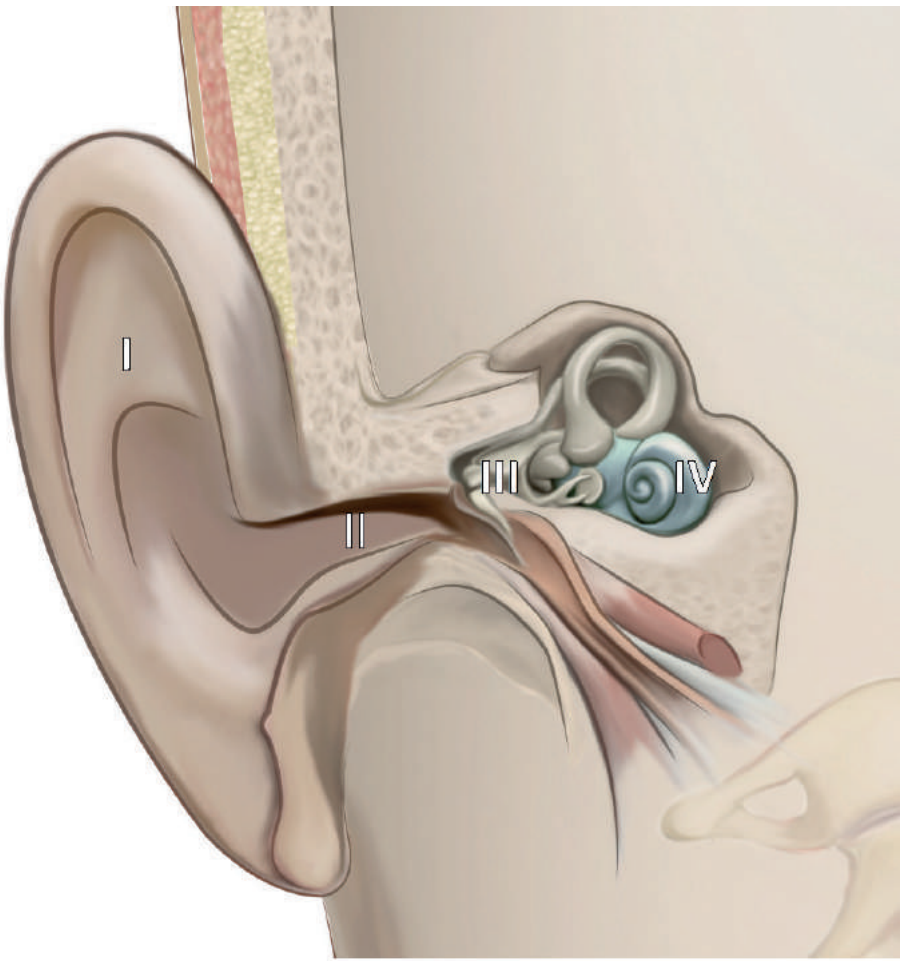

Figure 1: Anatomy of the ear: (I): Outer ear with pinna, (II) external auditory canal, (III) Middle ear, (IV) Inner ear (Cochlea) Image made available with courtesy of Oticon Medical AB (Askim, Sweden).

Bone conduction hearing

Besides air conduction hearing, bone conduction is an alternative pathway for hearing. Bone conduction vibrations are transferred through the skull. The vibrations can be transferred to the external ear canal, middle ear, directly to the cochlea and to the cerebrospinal fluid bypassing one or several parts of the outer and middle ear. Von Bekesy was one of the first to formulate mechanisms relating to bone conduction [1]. Several components have been proposed to facilitate/enable bone conduction hearing including [2]:

- Fluid inertia: Skull vibrations result in vibrations of the cochlea itself. Due to inertia of the cochlear fluid and fluid outlets (the oval and round window), the fluids can move causing a flow of cochlear fluid.

- Cochlear bone deformation: The vibration of the skull result in cochlear bone deformation or compression of the cochlear walls. These can result in cochlear fluid movement due to the asymmetry in scala vestibuli and scala tympani [3].
- Outer ear: Some parts of the external ear and middle ear are of importance for bone conduction as well. When the external ear canal is blocked (*e.g.* headphone) skull vibrations can enter the external ear canal which can result in direct stimulation of the ear drum via entrapped air.
- Middle ear: In the middle ear, the tympanum and annular ligament are attached to the stapes footplate. Both the ligament and tympanum are considered springs attached to the ossicles. When the bone of the tympanic cavity vibrates, the ossicles start moving due to this spring mechanism.
- Soft tissue: Soft tissue such as cerebrospinal fluid can conduct vibrations to the cochlea without direct bone transfer.

Hearing loss

In 2012 the World Health Organization estimated that 5.3% of the world's population is suffering from some form of hearing impairment [4]. Hearing loss can be divided into sensorineural hearing loss, conductive hearing loss or a combination of both, called mixed hearing loss. Sensorineural hearing loss should be regarded as the most common form of hearing loss which is usually associated with a deterioration of the cochlear hair cells. Conductive hearing loss is acquired by some outer or middle ear problem, which impairs the normal amplification function of the outer and middle ear (*e.g.* microtia, ear drum perforation, immobile or incomplete ossicles). In case of mixed hearing loss, both a conductive and sensorineural hearing loss attribute to hearing loss. In Single Sided Deafness (SSD), there is a complete unilateral lack of hearing. Treatment of hearing loss varies according to its nature and severity and may include middle ear surgery or the application of conventional air conduction hearing aids. In a specific category of patients (*e.g.* severe eczema of the hearing canal, sound distortion or social issues), these options can be unsatisfactory and bone conduction hearing may be used.

Bone anchored hearing systems

In 1977 Tjellström and Brånemark introduced the Bone Anchored Hearing System (BAHS), also known as the Bone Anchored Hearing Aid (BAHA), Bone Anchored Hearing Implant (BAHI) or Bone Conduction Hearing Implant (BCHI) [5] (Figure 2) . The BAHS consists of a titanium fixture implanted in the retro-auricular skull mounted with a skin penetrating abutment. A sound processor can be attached to the abutment which vibrates and transfers sound waves to the skull. In the last decades, the BAHS has become an accepted treatment option for subjects suffering from conductive hearing loss, mixed hearing loss or SSD[6].

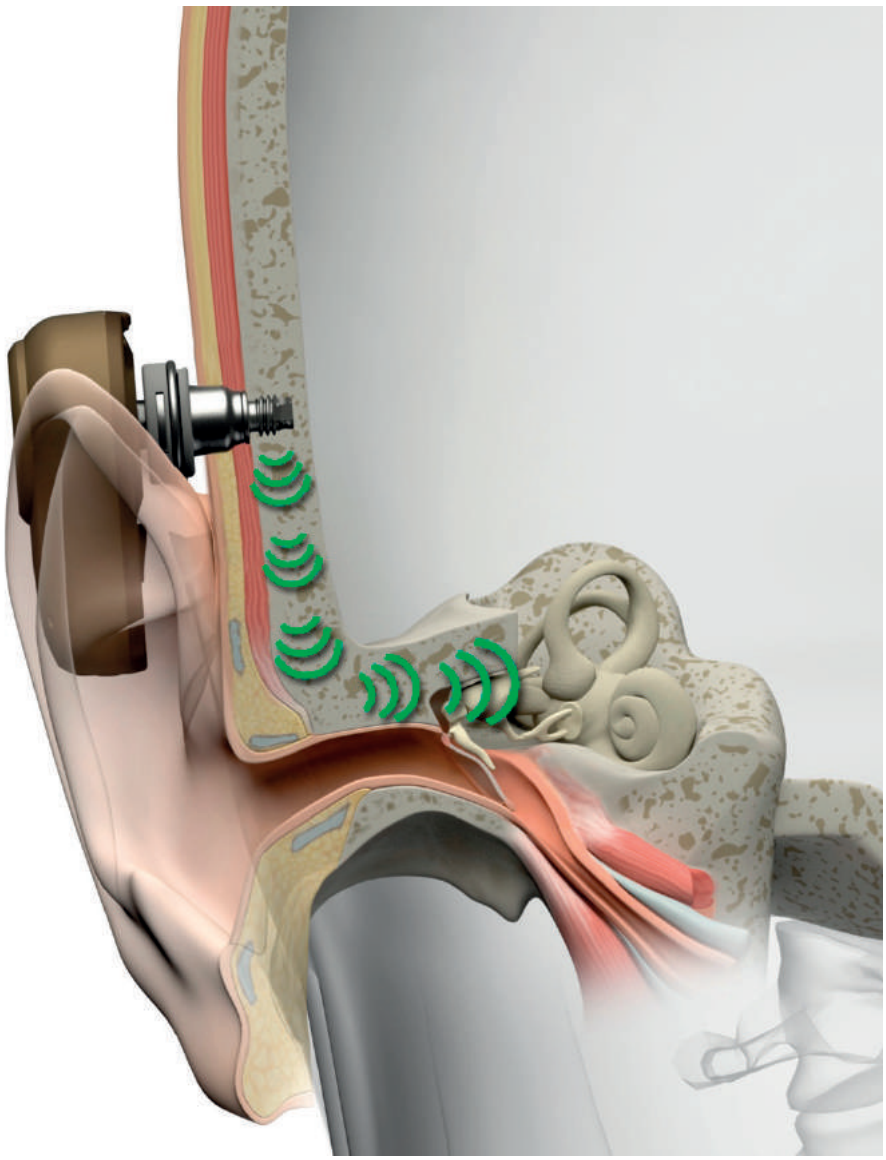

Figure 2: Example of Bone Anchored Hearing System: Sound waves are transferred intro vibrations by the processor to the bone anchored implant. The vibrations can be transferred to the middle and inner ear. Image made available with courtesy of Oticon Medical AB (Askim, Sweden).

To date, worldwide an estimated 200.000 BAHS surgeries have been performed[7]. Over time, the surgical technique, implant design and abutment design have changed. Soon after the introduction of the BAHS, Tjellstrom *et al.* observed that inflammation of the peri-abutment skin or soft tissue inflammation arised. Peri-abutment skin inflammation is currently perceived as one of the major complications related to the BAHS [8]. Several

factors, including surgical technique, abutment design, skin movements, immune responses and bacterial presence are believed to play a role in the etiology of inflammation. However, the role of each factor remains to be investigated. Tjellstrom *et al.* hypothesized that the skin movements around the immobile percutaneous BAHS was one of the causes for soft tissue inflammation[9]. To reduce movement of the implant as much as possible, several skin reduction techniques were introduced. In contrast to this general belief, van de Berg *et al.* showed that less invasive surgical techniques concerning the periabutment skin resulted in , less soft tissue complications [10]. Following this insight, Hultcrantz showed that the linear incision technique with soft tissue preservation led to favorable outcomes regarding wound healing, soft tissue reactions and cosmetics [11,12]. However, complications such as numbness, pain, soft tissue overgrowth, soft tissue inflammation and extrusion still occur. Less invasive surgical techniques are supposed to further improve outcomes. Several surgeons have experimented with punch only techniques which would alleviate the need for an incisional scar and raising the mucoperiosteal flap in the peri-implant surrounding area, thereby minimizing soft tissue damage [13,14]. In 2013, Oticon Medical (Askim, Sweden) sought to develop a punch-only technique to minimize soft tissue damage and improve surgical outcomes. This led to the introduction of Minimally Invasive Ponto Surgery (MIPS) in 2015 [15].

The first abutments were designed to have a rather sharp angle to the adjacent soft tissue. This sharp angle are believed to cause more soft tissue reactions. Therefore, the abutment have been refashioned to designs with a more curved angle in relation to the soft tissue. Additionally, surface properties are currently being modified. A Hydroxyapatite coated abutment was introduced [16]. To prevent adherence of bacteria, extra smooth abutments are currently being investigated as well. The hydroxyapatite coated abutment has been demonstrated to integrate with soft tissue [17]. The clinical outcomes of RCT investigating the Hydroxyapatite coated abutment are expected to become available in 2018 (NCT01796236). Results on a clinical survey study comparing a new smooth titanium abutment to the conventional titanium abutment showed no differences in clinical outcome [18]. The outcomes of these studies might be used to further understand the impact of both approaches.

In 1988 Holgers described these skin reactions according to the Holgers Index, which has become the most common used skin assessment scale for BAHS [19]. Holgers showed that bacteria can be found on the BAHS, including *Staphylococcus aureus* and *Coagulase Negative Staphylococci, Bacteriodes urealyticus, Proteus, Klebsiella, E. coli* and *Peptostreptococcum* [20]. Monksfield *et al.* found that bacteria can reside on BAHS as biofilms as well [21], which are particularly resistant to antibiotics. Moreover, biofilms cannot always be cultured using standard techniques. Additionally, the same group found that protein expression of various cytokines in the peri-abutment crevice fluid is higher in inflamed BAHS compared to non-inflamed BAHS using an immunoassay[22]. A prospective study, to compare cytokine expression and novel techniques which allow for improved characterization of biofilms could improve our understanding on soft tissue inflammation in BAHS.

Thesis outline

In this thesis, two separate parts are presented with an individual focus. The first chapters describe the clinical aspects related to BAHS. The second part describes more fundamental research questions related to BAHS implantation, cases of soft tissue inflammation and imaging.

Part I: Clinical outcomes

In **chapter 2**, the design of a multicenter randomized controlled trial to compare the MIPS technique to the linear incision technique with soft tissue preservation for BAHS is described. Explorative aims to gain further understanding on soft tissue inflammation are delineated.

In **chapter 3**, the 12-week follow-up results of the multicenter randomized trial described in chapter 2 are presented. MIPS seems to have some favorable outcomes compared to the linear incision technique with soft tissue preservation. However, implant losses are a concern warranting further study.

In **chapter 4**, a retrospective study to estimate the overall implant survival of all subjects having received a BAHS in Maastricht over the last 25 years is described. Risk factors for implant losses such as age, shorter implants and implantation after loss of a previous implant are described.

Part II: Understanding clinical outcomes

In **chapter 5**, the potential use of Cone Beam CT (CBCT) imaging in BAHS is described. *In vitro* factors that could influence the Implant Stability Quotient (ISQ), such as bone density and partial seating are studied. Using virtual markings, an estimate for partial seating is described. Additionally, 3D image analysis is presented as an option for qualitative assessment of BAHS seating.

In **chapter 6** a prospective study comparing cytokine expression of the peri-abutment skin at baseline, 12-week follow-up and during cases of inflammation is described and correlated to clinical factors such as the Holgers Index, smoking habits and pain scorings.

In **chapter 7** the microbiome of the BAHS is described in a prospective study using a molecular technique. The microbiome prior to implantation, at 12-week follow-up and during cases of inflammation is presented. Diversity, correlations within subjects over time, between subjects over time and specific species related to inflammation are described.

A case report for an explanted BAHS implant is presented in **chapter 8**. Two-year post-surgery, a BAHS was retrieved due to chronic pain complaints after abutment removal. Histology, micro-CT, Back Scattered Scanning Electron Microscopy, Raman spectroscopy and microbiology are employed to provide an overview of the implant. A possible explanation for chronic pain related to the BAHS is presented.

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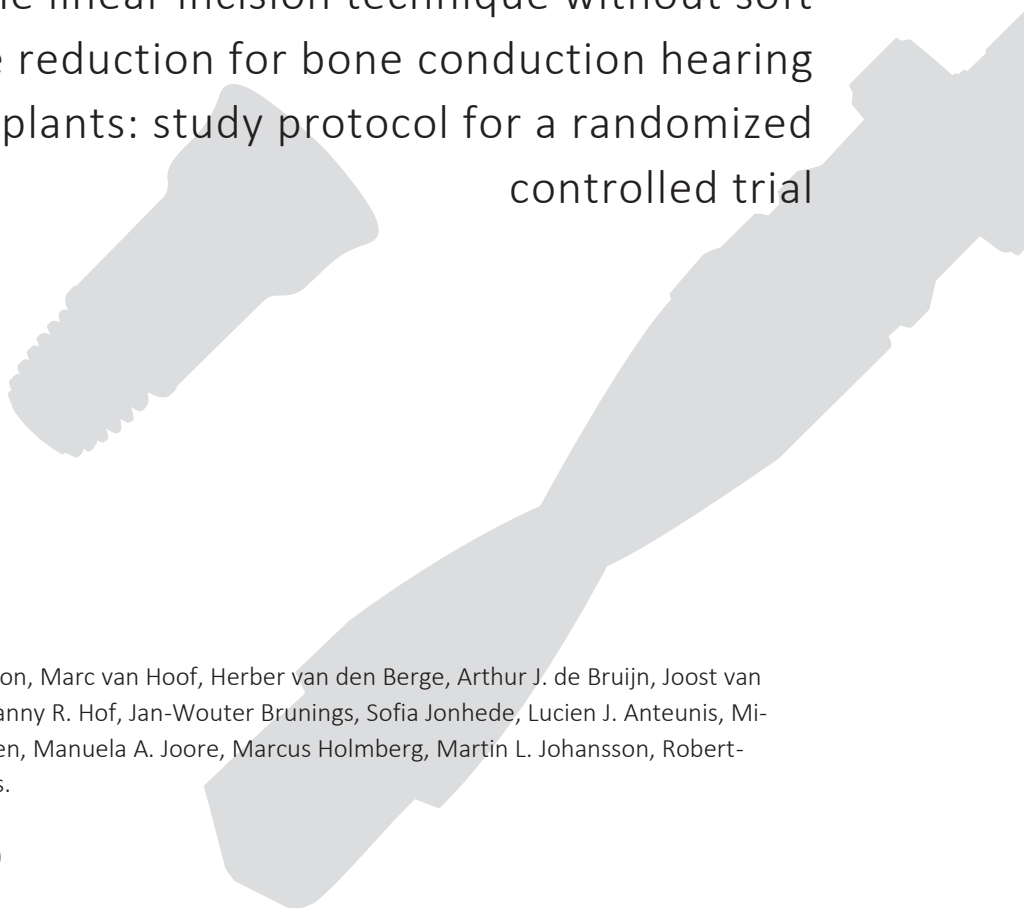
Part I

Clinical outcomes

Chapter 2

Minimally Invasive Ponto Surgery compared to the linear incision technique without soft tissue reduction for bone conduction hearing implants: study protocol for a randomized controlled trial



Tim G.A. Calon, Marc van Hoof, Herber van den Berge, Arthur J. de Bruijn, Joost van Tongeren, Janny R. Hof, Jan-Wouter Brunings, Sofia Jonhede, Lucien J. Anteunis, Miranda Janssen, Manuela A. Joore, Marcus Holmberg, Martin L. Johansson, Robert-Jan Stokroos.

Trials (2016)

Abstract

Background: Over the last years, less invasive surgical techniques with soft-tissue preservation for Bone Conduction Hearing Implants (BCHI) have been introduced such as the linear incision technique combined with a punch. Results using this technique seem favorable in terms of rate of Peri-Abutment Dermatitis (PAD), aesthetics and preservation of skin sensibility. Recently, a new standardized surgical technique for BCHI placement, the Minimally Invasive Ponto Surgery (MIPS) technique has been developed by Oticon Medical AB (Askim, Sweden). This technique aims to standardize surgery by using a novel surgical instrumentation kit and minimize soft tissue trauma

Methods: A multi-center randomized controlled trial is designed to compare the MIPS technique to the linear incision technique with soft tissue preservation. The primary investigation center is Maastricht University Medical Center. Sixty-two participants will be included with a two-year follow-up period. Parameters are introduced to quantify factors such as loss of skin sensibility, dehiscence of the skin next to the abutment, skin overgrowth and cosmetic results. A new type of sampling method is incorporated to aid in the estimation of complications. To gain further understanding of PAD, swabs and skin biopsies are collected during follow-up visits for evaluation of the bacterial profile and inflammatory cytokine expression.

The primary objective of the study is to compare the incidence of PAD during the first 3 months after BCHI placement. Secondary objectives include the assessment of parameters related to surgery, wound healing, pain, loss of sensibility of the skin around the implant, implant extrusion rate, implant stability measurements, dehiscence of the skin next to the abutment and esthetic appeal. Tertiary objectives include assessment of other factors related to PAD and a health economic evaluation.

Discussion: This is the first trial to compare the recently developed MIPS technique to the linear incision technique with soft tissue preservation for BCHI surgery. Newly introduced parameters and sampling method will aid in the prediction of results and complications after BCHI placement.

Trial registration: Registered at the CCMO register in the Netherlands on November 24th 2014: NL50072.068.14. Retrospectively registered on April 21st 2015 at ClinicalTrials.gov: NCT02438618. This trial is sponsored by Oticon Medical AB.

Keywords: MIPS, Bone conduction hearing implants (BCHI), soft tissue preservation, BAHA, Bone Conduction Device (BCD), Randomized Controlled Trial (RCT)

Background

The World Health Organization estimated that approximately 360 million people worldwide suffer from disabling Hearing Loss (HL) [1]. People with HL can often benefit from the use of hearing devices, such as hearing aids, but patients with *e.g.* conductive hearing loss cannot always profit from traditional hearing aids. In order to improve hearing for this group of patients, the bone anchored hearing aid (BAHA), also known as the bone conduction hearing implant (BCHI) was introduced in 1981 [2]. The BCHI consists of a titanium fixture implanted in the retro-auricular bone of the skull with an abutment that breaches the skin, so that a sound processor can be attached to it. The sound processor converts sound waves into vibrations via its transducer. These vibrations are conducted by the abutment to the titanium fixture and ultimately to the skull. The skull conducts these vibrations to both inner ears, bypassing any problems in the ear canal or middle ear [3]. BCHIs are currently considered a suitable treatment option for three groups of patients, namely patients with conductive HL, mixed HL or single sided deafness (SSD).

This technology has been reported to improve the quality of life for these patients, although the available data is limited [4,5]. Recently, measuring quality of life and well-being using the concept of capabilities has gained more interest [6]. It can be expected that interventions that entail placing a percutaneous implant to improve hearing, may influence an individual in more ways than just solely altering hearing. (*e.g*. the ability to participate in society versus the perceived disadvantages of these types of implants such as cosmetic and social concerns [7,8]). This perspective will be considered in this trial as well. Over the years, the BCHI has become an established treatment option with approximately 200.000 BCHI surgeries worldwide to date [9].

Although successful, the BCHI has complications. Known problems include skin inflammation, also known as peri-abutment dermatitis (PAD), pain, numbness of the skin around the implant, skin overgrowth and implant loss [10]. PAD is graded on a five point scale called the Holgers Index [11] and is perceived as the most common complication of BCHI use with an estimated occurrence of 16,1 - 38,1% among all recipients [10]. PAD is an inflammatory process that is presumed to be multifactorial and little is known about the precise etiology of this condition [12]. Shear stress on the skin around the abutment [2,13] sets on as the implant and abutment combination fixed to the skull is immovable. This may cause tearing of the skin (*e.g.* while turning on a pillow at night or during head and jaw movements). The implant and abutment are made of titanium which may elicit a foreign body response [12,14]. Also the formation of a biofilm on the abutment by bacteria that colonize the wound site due to persistent breach of the skin might play an important role [15,16]. Another side-effect of the BCHI is pain. This is frequently experienced by patients [17], however the exact cause for this, sometimes chronic pain, remains unknown [18–20]. Another condition which has anecdotally been observed is skin sagging, the presence of excess skin cranially to the abutment [21]. This might be

problematic as it can influence the sound processor coupling and the proper function of the processor.

Various strategies for reducing complication rates with BCHIs have been employed throughout the years. Traditionally, the skin surrounding the abutment was thinned, but Van de Berg *et al.* noted lower complication rates after BCHI surgery with a less invasive approach [20]. Since then, an even less invasive, single-stage procedure, where the soft tissue surrounding the abutment is left intact [22–25], has become one of the most common techniques used for BCHI surgery. Advantages of this tissue preserving approach include: less surgical procedure time, reduced numbness or pain and better cosmetic results [22,24,25].

The osteotomy preparation, assuring minimal trauma to the bone, space to maneuver the burr head and adequate cooling of the site as well as minimal trauma and displacement of the soft tissue, are all important factors when designing a procedure for installing a bone anchored percutaneous implant [26]. Furthermore, to achieve comparable results across surgeries, the variation in surgical technique introduced by different surgeons needs to be minimized. Based on local clinical practices and available tools, surgeons in different countries have started using punch-only surgical techniques for BCHI surgery [27–29]. The available surgical tools were not developed for this punch-only approach, presenting potential drawbacks such as soft tissue damage and insufficient irrigation. In pursuance of developing a standardized minimally invasive punch-only surgical kit and method, Oticon Medical AB (Askim, Sweden) started the design of instruments for a single stage BCHI surgical technique in 2013. The goal of this new Minimally Invasive Ponto Surgery (MIPS), was to optimize tissue preservation, minimize tissue trauma and provide a punch-only standardized surgical procedure with standardized surgical equipment aiming to eliminate surgical variability [26].

Previous BCHI clinical trials used non-validated outcome measurement scales, creating a need for validated alternatives. Most questionnaires and endpoints currently used to evaluate the BCHI from the perspective of the clinicians rather than that of the patients' [30]. We know that studies using non-validated scales are prone to risk of bias [31]. In contrast, studies that use reliable, systematic and validated outcome measures give the possibility to compare different trials and perform a meta-analysis [32]. Another limitation is that the number of prospective randomized controlled trials in the field is low [22,33–36]. Moreover, complications related to the BCHI can vary over time and may be missed by only assessing patients at fixed time points coinciding with preplanned trial visits. Important information is lost this way and incidence numbers might become under or overestimated. By not collecting all the information which is available, the ability to find a difference between two interventions also decreases. In this clinical trial, the amount of standard visits is decreased in favor of collecting more information during extra consultations in case of problems.

The decision to choose one intervention over the other in a health care system *with limited resources* depends on the associated (clinical) benefits but also on the incurred

additional costs or cost savings. As reviewed by Crowson *et al.* [4], only a few investigations addressed the cost-effectiveness of a BCHI intervention, resulting in uncertainty regarding cost-effectiveness. One retrospective study by Monksfield *et al.* has been executed in the United Kingdom that compared gain in quality-adjusted life years to BCHI related costs. Costs included: implantation surgery, post-surgical care, the first processor, annual check-up, processor maintenance and processor replacement costs after 3 years [5]. This study concluded that the BCHI is probably cost-effective. So far, no study included non-health care costs, such as loss of productivity, travel costs and out-of-pocket costs.

In this paper we describe the research protocol for a multicenter randomized controlled study comparing the new MIPS technique to the linear incision technique with soft tissue preservation [24,26] comparing the incidence of inflammation as primary objective. In this trial, revised parameter scales are introduced to quantify factors such as loss of skin sensibility, dehiscence of the skin next to the abutment, skin overgrowth and cosmetic results. These are modified to reduce the subjective interpretation and are intended to be validated. Exploratory outcome measures related to PAD such as skin biopsies bacterial swabs are collected. An economical evaluation is planned with quality of life approached from a capability perspective as well. The MIPS technique is hypothesized to result in a lower incidence of inflammation compared to the linear incision technique with soft tissue preservation.

Methods

Study design, ethics, setting and recruitment

This study is a sponsor initiated multicenter, open, randomized, controlled clinical investigation. This article has been drafted following SPIRIT guidelines [37]. Three hospitals in the Netherlands are currently recruiting participants for this study: Maastricht University Medical Center (MUMC+), ZiekenhuisGroep Twente (ZGT) and Medisch Centrum Leeuwarden (MCL). MUMC+ is an academic teaching hospital. ZGT and MCL are general hospitals. This multicenter study is performed in accordance with the Declaration of Helsinki [38], has been approved by the ethics committee of MUMC+ (NL50072.068.14) and has been registered at clinicaltrials.gov (trial number: NCT02438618). The local ethics committees of ZGT and MCL approved the local execution of this trial. A total of sixty-two participants will be recruited at the ear, nose and throat outpatient departments by the (local) researchers. Patients are considered eligible for participation [22,39] *(I)* if they will undergo unilateral BCHI surgery and *(II)* when they are ≥ 18 years of age. Participants will be excluded from participation in case of *(I)* a history of immunosuppressive disease, *(II)* usage of systemic immunosuppressive medication, *(III)* bilateral BCHI placement, *(IV)* relevant dermatological disease (e.g. psoriasis, severe eczema), *(V)* participation in other studies, and *(VI)* when no suitable site for a 4 mm wide implantation during surgery is found.

Study interventions and allocation

All participants will undergo single stage surgery to receive a 4 mm Ponto wide implant with mounted abutment (Oticon Medical AB, Askim, Sweden), which will be performed by an experienced ENT surgeon. Four abutment lengths are available: 6, 9, 12 and 14 mm. After written informed consent, participants will be randomized to the test group (MIPS technique [26]) or the control group (linear incision technique with soft tissue preservation [24]) in the order in which they enter the study. Skin-thickness is a possible factor that could influence inflammation, but this is measured during surgery, making it impossible to stratify for skin-thickness prior to surgery. Considering that men are known to have significantly thicker skin than women [40], group allocation is stratified for gender. Researchers will randomize each subject using randomization software (Statistiska konsultgruppen, Gothenburg, Sweden), which will be performed in each research center independently in a 1:1 ratio for the test and control group stratified for gender until the total number of sixty-two participants has been reached. Due to clear differences in surgical techniques, it is impossible to blind the surgeon, researcher or subject. In short both surgical techniques are described here:

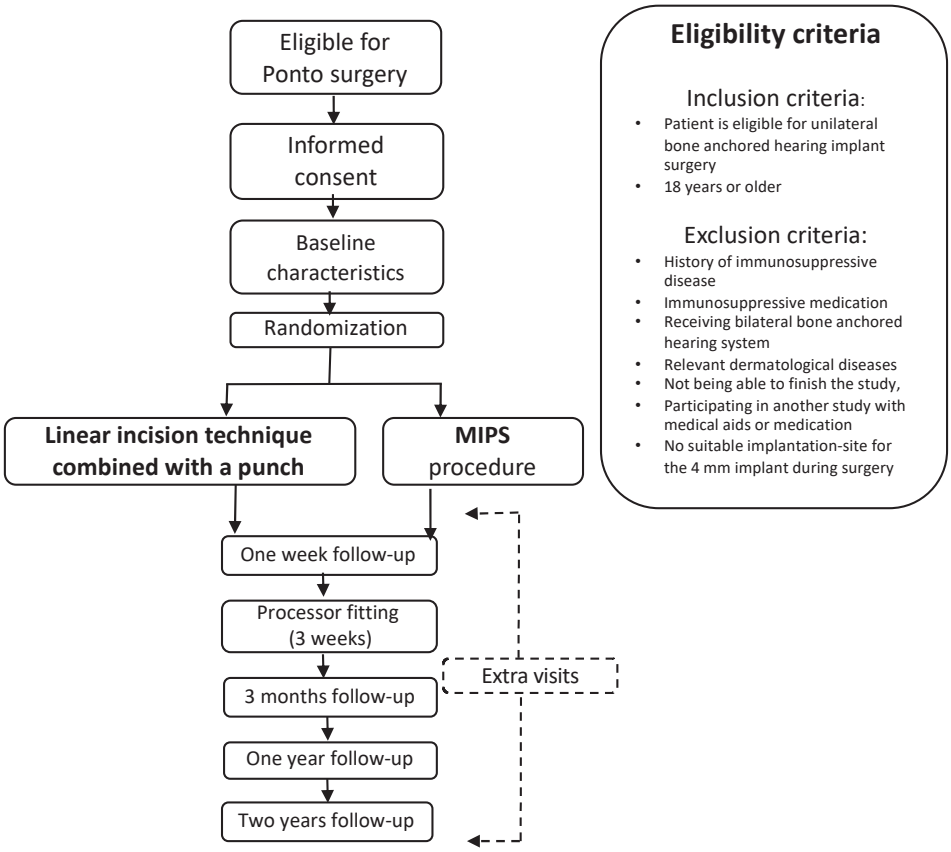


Figure 1: Study design and study flow-chart.

Control group (Linear incision technique with soft tissue preservation) [24,41]
The intended implant position is marked (Figure 2A). *(I)* A Retro-auricular linear incision is made down to the periosteum that is subsequently opened to expose the periosteum posterior to the incision line (Figure 2B). *(II)* At the intended implant site, a central area of periosteum is removed. *(III)* An initial 3 mm deep hole is created using a guide drill. *(IV)* If there is still bone at the bottom of the initial hole, it is deepened to 4 mm using the same drill by removing a spacer. *(V)* To prepare the initial hole for implant insertion it is widened with a countersink drill. *(VI)* The implant with mounted abutment is installed with a torque setting of 40-50 Ncm. *(VII)* The skin is retracted over the abutment and dermal sutures are placed. *(VIII)* The abutment is recovered by punching the skin with a 5 mm punch. *(IX)* A healing cap is attached to the abutment and a gauze drenched in an antibiotic ointment is applied.

Test group (MIPS technique) [26,42]

The intended implant position is marked (Figure 2A). *(I)* An incision is created with a 5 mm punch at the intended implant site (Figure 2C). *(II)* The periosteum and remaining soft tissue around the incision hole are removed with a raspatorium. *(III)* The cannula is inserted at the surgical site. *(IV)* A 3 mm hole is created initially with the cannula guide drill. *(V)* If there is bone at the bottom of the 3 mm hole, it is deepened to 4 mm using the same drill by removing a spacer. *(VI)* To prepare the initial hole for implant insertion, it is widened with the cannula widening drill. *(VII)* The cannula is removed and subsequently the implant with mounted abutment is installed with a torque setting of 40-50 Ncm. *(VIII)* To help estimate complete insertion of the implant, an installation indicator is attached to the abutment inserter, which makes it possible for the surgeon to count the number of rotations. This step was added during the course of the study. *(IX)* A healing cap is attached to the abutment and a gauze drenched in an antibiotic ointment is applied.

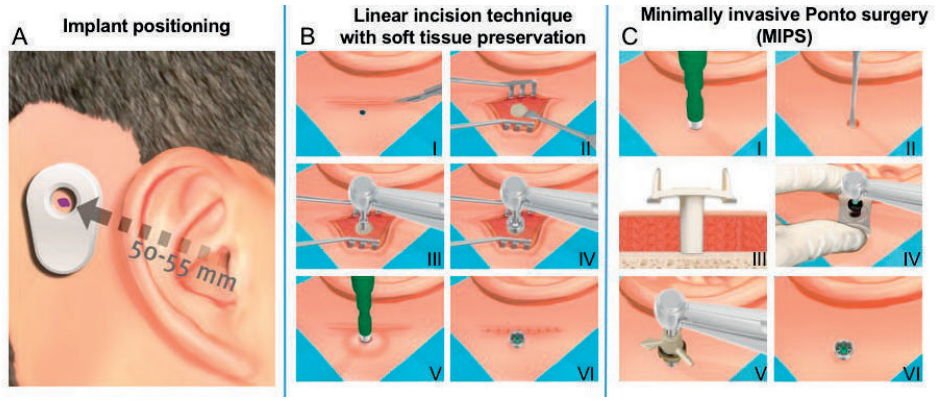


Figure 2: Surgical implantation techniques. (**A**) Implant positioning. (**B**) Schematic presentation of the linear incision technique with soft tissue preservation. *(I)* Linear incision. *(II)* Opening of skin. *(III)* Initial hole drilling. *(IV)* Countersink drilling. *(V)* Eccentric skin punch to uncover abutment. *(VI)* Result. (**C**) Schematic presentation of Minimally invasive Ponto surgery (MIPS) technique. *(I)* Incision hole. *(II)* Removal of periost and soft tissue. *(III)* Placement of cannula. *(IV)* Drilling procedure (cannula guide drill and cannula widening drill). *(V)* Implant placement with the insertion indicator. *(VI)* Result.

Follow-up

Follow-up visits for all participants are scheduled at 9 days, 21 days, 3 months, one year and two-years post-surgery (Figure 1). During this period all extra consultations are captured in high detail. The consultations are largely comparable to standard visits (Table 1). By compiling extra consultation visits and regular follow-up visits, we can present a more accurate estimate of common complications that can occur at any time point. Outcomes

Primary outcome

The primary outcome of this study is the incidence of inflammation (episodes of PAD) between surgery and 3 months post-surgery. During each visit, the peri-abutment skin is graded based on the Holgers Index. The Holgers Index is a five-point scale described by Holgers [11]: "0 No irritation; 1 Slight redness; 2 Red and slightly moist tissue, no granuloma formation; 3 Reddish and moist; sometimes granulation tissue; 4 Removal of skin-penetrating implant necessary due to infection" [11]. In this study, inflammation has been defined as the occurrence of a Holgers Index ≥ 2 as this often requires substantial treatment (e.g. systemic antibiotics or local intervention).

Table 1: Schedule of endpoint assessments Follow-up visits are planned post-surgery. Pain assessment: Score for pain around the implant / radiating pain / headache associated with implant. N.A.: Not applicable. Cosmetic result scores: Score for natural skin position, baldness, scarring, skin color, indentation, overall cosmetic score. Gray chart indicates measurements assessed follow-up visits/extra consultations with a Holgers Index score ≥ 2. *: Outcome measurements obtained at Maastricht University Medical Center only. ˇ: Only after explicit additional informed consent.

Outcomes	Description	Units	Baseline	Surgery	9 Days	3 Weeks	3 Months	1 Year	2 Years	Extra consultation	Any visit Holgers Index ≥ 2
Primary outcome											
Incidence of inflammation	Scoring according to the Holgers Index.	0-4			x	x	x	x	x	x	
Secondary outcomes											
Surgical procedure time	Time during surgery and time in operation theater.	Minutes		x							
Wound healing time	Time to initial healing.	Yes/No/ Partial			x	x	x				
RFA: ISQ measurements	Horizontal and vertical ISQ values	0-100		x	x	x	x	x	x	x	
Pain	Pain assessment	0-10			x	x	x	x	x	x	
Soft tissue height/ overgrowth	Distance from abutment to skin in four quadrants.	Millimeters			x	x	x	x	x	x	
Dehiscence	Presence of dehiscence.	Yes/No			x	x	x	x	x	x	
Skin sagging	Presence of skin sagging in four quadrants.	Yes/No			x	x	x	x	x	x	
Extrusion	Implant extrusion at any time point.	Yes/No			x	x	x	x	x	x	

Outcomes	Description	Units	Baseline	Surgery	9 Days	3 Weeks	3 Months	1 Year	2 Years	Extra consultation	Any visit Holgers Index ≥ 2
Loss of skin sensibility	Loss of sensibility from abutment to the most outer point.	Millimeters			x	x	x	x	x		
Cosmetic results	Cosmetic result scores.	0-10					x	x	x		
Tertiary outcomes											
Quality of life questionnaires	HUI3, APHAB and ICECA.	N.A.	x					x	x		x
Exploratory outcomes											
Skin position	Photographs with markers. pre- and post- surgery.	Distance measure		X*							
Skin movements	Photographs in different head positions.	Distance measure.			X*						
Bacterial content	Bacterial swabs (IS-PRO).	IS-Lengts, quantify.		X*			X*				X*
Cytokine expression	Peri-abutment biopsies.	N.A.		X*			X*				X*ˇ
Diagnostic photography	High quality photographs of BCHI.	N.A.	x	x	x	x	x	x	x	x*	

Secondary outcomes

Secondary outcomes include surgical procedure time, wound healing, presence of dehiscence after surgery, soft tissue height, loss of skin sensibility, pain, cosmetic results, Implant Stability Quotient (ISQ) values and extrusion rate (Table 1).

Surgical procedure time consists of the length of surgery (from the incision until the placement of the healing cap) and the total time spend in the operation theatre by the subject (entering theatre until leaving theatre) in minutes. Both timings are measured using a stopwatch and are included because the preparation and post-surgical care can differ. Wound healing is evaluated during standard follow-up visits and can be graded as complete, partial or incomplete. The presence of dehiscence is evaluated at all visits. Soft tissue height and overgrowth is assessed by measuring the distance from the top of the abutment to the skin in four quadrants. Numbness is measured as the start of sensibility from the abutment to the most outward diameter. Pain is assessed for three separate domains including pain directly around the abutment, radiating pain and headache that is related to the BCHI. Pain is graded in a 10-point scale with a scale of 0 representing "no pain" to 10 representing "the worst pain imaginable". Cosmetic results are assessed by the surgeon using several properties which were thought to be influenced by the surgical technique. These included the folding of the skin around the abutment, baldness, scarring, skin color, indentation and an overall cosmetic score as assessed by the surgeon and subject. All results are graded on a 10-point scale and compared to the contralateral side if applicable. ISQ values are obtained with the Ostell ISQ equipment (Ostell, Gothenburg, Sweden) by mounting a Smartpeg Type 55 on the abutment and obtaining 2 perpendicular ISQ values at all visits. All cases of abutment exchanges and implant extrusion are noted (time of extrusion, reason if known and subsequent action).

Tertiary outcomes

Quality of life and economic evaluation

Three questionnaires are used to evaluate the impact on hearing specific and generic quality of life and capabilities. The questionnaires are filled in at baseline, at one-year follow-up and at two-year follow-up. The Abbreviated Profile of Hearing Aid Benefit (APHAB) is a 24-item self-assessment, disability-based inventory, designed for hearing related quality of life [43]. Each item is assessed in both the unaided and aided situation. The Health Utilities Index Mark 3 (HUI 3) is a preference-based system for measuring generic health-related quality of life , consisting of 17 questions [44]. It provides descriptive evidence on multiple dimensions of health status, including overall health and several health dimensions. These dimensions include: vision, hearing, speech, ambulation/mobility, pain, dexterity, self-care, emotion and cognition. Each dimension has 3-6 discriminatory levels, making it sensitive to health changes induced by interventions [45]. The ICEpop CAPability

measure for Adults (ICECAP-A) was designed [46] to measure capabilities [47]. Capabilities represent the "freedom" of an individual to "achieve" a certain functioning, without the need to have actually achieved this [48]. This 5-point questionnaire has been translated and validated in Dutch [49]. It specifically assesses the capabilities attachment, security, enjoyment, role and control. These questions include aspects as independence, dignity, comfort and social interaction, which might be influenced by hearing loss and subsequent interventions. A last tertiary objective of this study is a full economical evaluation after data has been collected for 1 year as we assume that one-year after surgery, the complication rate, benefits, satisfaction and BCHI processor usage are stable in both treatment groups. Cost will be identified at each study visit using the Case Report Form (CRF) which is designed to identify costs. We will perform a cost-utility analysis, with the quality adjusted life year calculated from the obtained HUI 3 scores as the outcome. The analysis will be executed using a societal perspective. The evaluation will be performed according to the standards and guidelines of the International Society for Pharmaco-economics and Outcomes Research (ISPOR) [50].

Exploratory outcomes

Skin displacement

High resolution photographs (MUMC+: Nikon D800E, NIKON CORPORATION, Tokyo, Japan) with an additional lens (Nikon AF-S VR Micro-Nikkon 105mm f/2.8G IF-ED, NIKON CORPORATION, Tokyo, Japan); ZGT and MCL: iPhone 6 (Apple Inc, Cupertino, California, USA) are collected at surgery and follow-up visits. These photographs can be used to evaluate the peri-abutment skin over time and to assess the skin movability in relation to movements of the head and jaw using standardized skin markings. These photographs will also be used to study the validity of the Holgers Index and to investigate peri-abutment dermatitis. In addition, the minimal and maximal size of the gap between the abutment and skin is measured in all quadrants.

Etiology of Peri-Abutment Dermatitis

These outcome measures are obtained at MUMC+ only. Skin biopsies of the implantation site are obtained during surgery, at three months post-surgery and during episodes of inflammation if participants have explicitly provided an additional written informed consent for this procedure. RNA will be extracted from the biopsies and subsequently cDNA will be synthesized. We intend to determine mRNA expression of fifteen selected genes (Table 2) using Real-Time Reverse Transcription Polymerase Chain Reaction. Pre-surgical mRNA expression (baseline) and post-surgical expression will be compared. Specific profiles related to inflammatory responses, tissue remodeling, vascularization and bacterial infection will be assessed.

Bacterial swabs of the abutment, peri-abutment skin and contralateral skin are collected during the same time points. The bacterial content will be evaluated with IS-pro™,

a novel 16S-23S rDNA interspace (IS)-region-based profiling method [51]. This method is devised to enable high-throughput molecular profiling of any microbiota. The combined data sets will be used to study peri-abutment dermatitis and validate the Holgers Index.

Table 2: Overview of cytokines

Inflammatory mediators	TGF- ß MIP-1α	Microbial infection
IL-1ß		TLR-2
IL-6	Tissue remodeling	TLR-4
IL-8		
TNF-α	MMP-9	Vascularization
IL-17	TIMP-1	VEGF-A
IL-10	COL1 α1	FGF-2

IL: Interleukin, TNF-α: Tumor necrosis factor alpha, TGF- ß: Transforming growth factor beta, MIP-1 α: Macrophage inflammatory protein 1 alfa, MMP-9: Matrix metalloproteinase 9, TIMP-1: Tissue inhibitor of metalloproteinase 1, COL1 α1: Collagen, type 1, alpha 1, TLR: Toll-like receptor, VEGF-A: Vascular endothelial growth factor A, FGF-2: Basic fibroblast growth factor-2.

Analysis

Sample size calculation

The incidence of inflammation, a Holgers Index ≥ 2, between surgery and 3 months post-surgery will be compared between both groups. The sample size is calculated using the concept of effect size (ES) as presented by Lerman and Cohen [52,53]. The proposed test concerns a two sample test for binomial proportions which is equivalent to a Yates corrected Chi Square test for a 2x2 contingency table. When taking into account a type 1 error level (α) of 0.05 and a power (1-β) of 0.8, the required sample size per group is: $n = 2(z\alpha + z\beta)^2 / ES^2 = 2(1.65 + 0.84)^2 / ES^2 = 12.4 / ES^2$. Assuming an ES index of *medium to large* with 'h' of 0.65 to discriminate between two proportions, the sample size is: $n = 12,4/h^2 = 12,4/ 0.65^2 = 29.4$. With an expected 5% drop-out rate, 31 participants are needed per group, resulting in a total sample size of 62 participants.

Statistical analysis

This study is designed to include several endpoints including short term results (3 months follow-up), long term results (2 years follow-up) and an economic evaluation. Short term results will be evaluated after all participants have reached three months follow-up and will describe the primary endpoint and secondary endpoints between surgery and 3 months follow-up. The primary endpoint will be described by comparing the proportions of inflammation between surgery and three months follow-up using a chi-square test. Long term

results will also be used for the economic analysis. Prior to analysis, a statistical analysis plan (SAP) will be created describing the method of analysis specifically for each endpoint.

Safety

Cases of adverse events or device deficiencies will be recorded in the CRF. All cases of Serious Adverse Events (SAE) will be recorded in the corresponding CRF as well and subsequently reported to the sponsor and responsible regulatory committees. The recorded events will be incorporated in the applicable study results.

Study management, oversight and publication.

The study is monitored by Oticon Medical AB in conjunction with TFS (Zaltbommel, The Netherlands). Due to the low risk classification of the investigated procedures, a data monitoring committee was not deemed necessary. Data handling will be conform to Dutch legislation. Source data is contained in the original records (the electronic patient dossier), original forms (questionnaires) and CRF. Data will be kept for 15 years. A data management team, consisting of the principal investigator, researchers from the coordinating center and representatives from the sponsor are established to oversee and manage data collection. Any collected human tissue will be adequately disposed of after analysis. Insurances are provided for all participants in accordance with Dutch legislation. Regular care will continue if a subject has finished follow-up or if a participant withdraws from the study. The results of this study will be submitted to peer reviewed journals without any publication restrictions by the sponsor.

Protocol amendments

After initial approval (protocol version 1.2 dated 04-11-2014), the study site opened for accrual on 1st December 2014. So far, the ethics committee has approved two substantial amendments to the study protocol. In June 2015, an amendment (protocol version 1.4 12-05-2015) was approved to increase the total number of participants from 42 to 62, resulting in a decrease of the ES from 0.8 to 0.65 with 80% power to discriminate between two proportions. In the same amendment, in order to include sufficient participants, the study was expanded from a single center study to a multicenter study. In October 2015, the second amendment (protocol version 1.5 11-08-2015) was approved to include MIPS surgical equipment update, based on feedback after the first surgeries. The alterations in MIPS surgical package included shortening of the cannula and the addition of wings in order to increase the grip and stability of the cannula. The guide drill was also modified, resulting in a wider drilling hole making it easier to manually feel the drilling hole with the widening drill. An installation indicator was also developed, to assist in visual feedback for insertion completeness by counting the number of rotations during insertion. These updates were incorporated as MIPS became available to multiple centers around the

world and surgeons provided feedback in relation to their individual outcomes. These updates are not expected to affect the primary endpoint in a significant way, hence no change in the number of participants was necessary for this amendment. The change in the surgical procedure might influence secondary outcome measures (e.g. surgery time, risks on complications such as incomplete insertion) to a limited extend. All MIPS surgeries will be analyzed as one pooled treatment group but the amount of participants per MIPS version will be reported on.

Discussion

In the design of this prospective multicenter trial, a total of 62 participants are intended to be included and followed for a period of 2 years. In this trial the MIPS procedure (designed to reduce tissue trauma, standardize surgery and alleviate the need for an incisional scar [26]) is compared to the soft tissue preservation technique [23,24], which can be regarded as the conventional method. The incidence of PAD (Holgers Index ≥ 2) between surgery and three months follow-up is the primary outcome measurement. Adapted measures scales describing loss of skin sensibility, cosmetic results and skin height are introduced. Additionally, various exploratory measurements including skin biopsies, bacterial profile and skin movement are incorporated in this study. This exploratory data might allow to conduct correlative and comparative analyses between the onset of PAD, a changed gene expression and the presence of different bacterial species. This might help in understanding the multifactorial role bacteria, the immune response and skin movements play in the etiology of PAD.

One of the objectives of this investigation is to collect biopsies and photographs to explore the incidences of inflammation. The latter will also be used to validate the Holgers Index or, if that is not achieved, to create a new scale for which an internal validation will then be available. Newly introduced outcome measures such as loss of sensibility, cosmetic outcomes, pocket size, skin height and skin sagging have not yet been validated. These scales are designed to include as little as possible subjective interpretation (*e.g*. using millimeters and predefined quadrants). Also because every subject will be photographed at every visit, the interpretation of the Holgers Index score can be justified post-hoc and the validity can be further investigated in the future.

Another important aspect this study addresses is the timing and frequency of relevant information sampling. The occurrence of complications may be missed while following the standardized time points (*e.g*. patients do not only show infections at predetermined visits). Variable sampling over time will possibly allow for a more accurate assessment of the duration and incidences of complications such as PAD, sensibility loss and pain over time. The use of Areas Under the Curve (AUC) is proposed here as a solution for incorporating information available from these extra consultations. We assume that and encourage participants to visit their ENT surgeon if they experience a complication (*e.g.*

inflammation or pain). By incorporating episodes of complications, the complication and its burden can be assessed over time. This approach has been estimated to result in an increased power without having to increase subject numbers. In addition, a more accurate representation of complications over time can possibly be achieved.

Besides conventional and hearing specific quality-of-life approaches (*e.g*. HUI3 and APHAB) this study also uses the ICECAP questionnaire that focuses on the wellbeing of a participant in a broad perspective. In the field of BCHI and related technologies, it is plausible that interventions have more benefits than just health gains or hearing improvements. These devices and interventions might also increase the autonomy, freedom to achieve or develop and impact on social interaction. These factors are important in the perspective of a person as a whole [54–56] and are currently overlooked by most questionnaires. Potentially, these factors may be playing an important role in cost-utility or effectiveness. Moreover, BCHI recipients consist out of a diverse complex population that might broadly differ in experienced benefit (*e.g.* SSD subjects [57]). Arguably, it might turn out that the ICECAP adds an important new dimension to consider with the availability of transcutaneous solutions as well. This might add a new perspective to the current complexity of the decision making process surrounding BCHI placement (many different device choices with highly different profiles), reimbursement policies, complications and patient preferences.

Limitations

Due to the differences in surgical procedures it is impossible to perform a blinded trial. The surgical wound created is evident for the allocated intervention to the clinician and the patient. The new intervention could potentially lead to unexpected events, as both the surgical approach and surgical tools have been modified extensively. To identify possible issues, (serious) adverse events will be qualitatively assessed to allow for a correct identification of differences and potential drawbacks. Although several methods have been implemented to maximize the collection of information on trial participants to increase the power of this trial, the small sample size in this study remains to be a major drawback.

Trial status

Recruitment started in December 2014 and is currently still ongoing. The predicted study completion date is August 2018. Short term results are expected in the last quarter of 2016.

Abbreviations

AUC Area Under the Curve; APHAB Abbreviated Profile of Hearing Aid Benefit; BAHA Bone Anchored Hearing Aid; BCHI Bone Conduction Hearing Implant; CRF Case Report Form ES Effect Size; HL Hearing Loss; HUI3 Health Utilities Index Mark 3; ICECAP-A ICEpop CAPability measure for Adults; ISQ Implant Stability Quotient; MCL Medisch Centrum Leeuwarden; MIPS Minimally Invasive Ponto Surgery;

MUMC+ Maastricht University Medical Center; PAD Peri-Abutment Dermatitis; SAE Serious Adverse Event; SSD Single Sided Deafness; ZGT ZiekenhuisGroep Twente

Declarations

Ethics approval and consent to participate

This multicenter study has been approved by the ethics committee of MUMC+ (NL50072.068.14). The local ethics committees of ZGT and MCL approved the local execution of this trial.

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Competing interests

T.C., M.v.H. and R.S. are supported with a research grant from Oticon Medical AB. M.J. and S.J are employed at Oticon Medical AB. M.H. was formerly employed at Oticon Medical AB. M.v.H. and R.S. have previously received a research grant from Cochlear Bone Anchored Solutions AB, Mölnlycke, Sweden.

Authors' contributions

T.C is involved in the execution, coordination and analysis of the study. M.v.H. is involved in the protocol design, execution, coordination and analysis of the study. T.C. and M.v.H. drafted the first version of the manuscript. H.v.d.B and A.d.B. are involved in the study execution and coordination of the peripheral study centers. J.v.T., J.H and J.W.B. are involved in the execution of the study. M.J. and S.J. are involved in the study design, coordination and analysis. M.H. was involved in the study design. M.A.J., A.J. and L. A. are involved in the design and analysis of the study. R.S. is involved in the design, execution, coordination and analysis of the study.

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Chapter 3

Minimally Invasive Ponto Surgery Versus the Linear Incision Technique With Soft Tissue Preservation for Bone Conduction Hearing Implants: A Multicenter Randomized Controlled Trial

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Abstract

Objective: To compare the surgical outcomes of the Minimally Invasive Ponto Surgery (MIPS) technique with those of the linear incision technique with soft-tissue preservation for bone-anchored hearing systems (BAHS)

Design: Sponsor-initiated multicentre, open, randomised, controlled clinical trial

Setting: Maastricht University Medical Centre, Ziekenhuisgroep Twente and Medisch Centrum Leeuwarden, all situated in The Netherlands

Participants: Sixty-four adult patients eligible for unilateral BAHS surgery

Interventions: Single-stage BAHS surgery with 1:1 randomisation to the linear incision technique with soft-tissue preservation (control) or the MIPS (test) group

Primary and secondary outcome measurements: Primary objective: compare the incidence of inflammation (Holgers Index ≥ 2) during 12 weeks' follow-up after surgery. Secondary objectives: skin dehiscence, pain scores, loss of sensibility around the implant, soft-tissue overgrowth, skin sagging, implant extrusion, cosmetic results, surgical time, wound healing and Implant Stability Quotient (ISQ) measurements.

Results: Sixty-three subjects were analysed in the Intention-to-treat population. No significant difference was found for the incidence of inflammation between groups. Loss of skin sensibility, cosmetic outcomes, skin sagging and surgical time were significantly better in the test group. No statistically significant differences were found for dehiscence, pain and soft-tissue overgrowth. A non-significant difference in extrusion was found for the test group. The ISQ was statistically influenced by the surgical technique, abutment length and time.

Conclusion: No significant differences between the MIPS and the linear incision techniques were observed regarding skin inflammation. MIPS results in a statistically significant reduction in the loss of skin sensibility, less skin sagging, improved cosmetic results and reduced surgical time. Although non-significant, the implant extrusion rate warrants further research.

Keywords: MIPS, Bone-Anchored Hearing, BAHS, Holgers Index, Surgical outcomes, Minimally Invasive Ponto Surgery, Surgical technique; Bone conduction, Hearing loss, Soft tissue reactions, Tissue preservation

Introduction

The bone-anchored hearing system (BAHS) has become an accepted treatment option for subjects suffering from various types of hearing loss, such as conductive or mixed hearing loss or single-sided deafness, who are unable to benefit from conventional therapies, such as air conduction hearing aids or reconstructive middle ear surgery (1,2). It consists of a retro-auricular-placed titanium implant mounted with a percutaneous abutment, to which a sound processor can be attached. The implant integrates with the skull through a process of osseo-integration. The sound processor receives sound, converts it to vibrations and uses the skull as a conductive material to transmit it to the cochlea directly, thereby bypassing the ear canal and middle ear (3,4).

One concern when it comes to the BAHS is the skin around the implant. For the BAHS, this soft-tissue status is commonly assessed using the five-grade Holgers Index (5). Peri-abutment inflammation, defined as a Holgers Index of 2 or above, is the most common complication of BAHS and it can be associated with pain and discomfort (6,7). Other complications related to BAHS include pain, numbness of the skin adjacent to the implant, soft-tissue overgrowth and implant extrusion (6,8).

To improve outcomes, both the implant design and the surgical technique have evolved over the last few decades (9-11). The design of the skin-penetrating abutment has been refined from an angulated sharp design to curved or cylindrical alternatives (11,12). Soon after the introduction of the BAHS, it was hypothesised that adverse soft-tissue reactions occur as a result of skin movements adjacent to the skin-penetrating abutment (13). This led to the development of surgical techniques with soft-tissue reduction to minimise skin movements. In contrast to this hypothesis, Van de Berg *et al.* demonstrated that less invasive surgical techniques produced better results (14). Following this, a linear incision technique without any soft-tissue reduction was introduced, further improving outcomes, and it is currently the most advocated technique (9,10). However, raising a mucoperiosteal flap is associated with a degree of tissue damage and discomfort for the patient and it requires more surgical work such as suturing. As a result, there is a need to improve the surgical technique to further diminish adverse soft-tissue events. It is suggested that, by leaving the soft tissue and vascular supply surrounding the percutaneous abutment intact, the prerequisites for effective wound healing would be largely retained (10). To this end, surgeons have attempted a punch-only technique (15-17). This would obviate the need for an incision, reduce procedure time and clinical work load, with the aim of minimising postoperative complications such as numbness, pain, swelling, infection and dehiscence, as well as possibly reducing costs. Until now, a standardised method and tools to perform the punch technique for BAHS have been lacking. Recently, the Minimally Invasive Ponto Surgery (MIPS) technique was introduced by Oticon Medical AB (Askim, Sweden) to address this problem (18,19). This surgical procedure is a punch-only technique performed with a specially designed surgical kit.

This is the first multicentre, randomised, controlled trial to compare the MIPS technique with the linear incision technique with soft-tissue preservation. In both groups, Ponto Wide implants (Oticon Medical AB) with mounted abutments were used, resulting in a design evaluating only the surgical technique. Here we report the surgical outcomes after three months' follow-up.

Methods

Study design

The study protocol of this multicentre, randomised, controlled trial has previously been published (20). Maastricht University Medical Centre (MUMC+), Ziekenhuisgroep Twente (ZGT) and Medisch Centrum Leeuwarden (MCL), all situated in The Netherlands, participated in the performance of the trial. Adult patients (above 18 years) who were eligible to undergo a unilateral BAHS were asked to participate in this trial. The exclusion criteria were *(I)* a history of immunosuppressive disease, *(II)* use of systemic immunosuppressive medication, *(III)* bilateral BAHS placement, *(IV)* relevant dermatological disease (e.g. psoriasis, severe eczema) and *(V)* participation in other studies. If a suitable site for a 4-mm implantation was not found or if the bone quality was assessed as being insufficient, the subject was regarded as early termination and excluded during surgery.

Randomisation and blinding

Enrolled subjects were allocated consecutively to the test group (MIPS technique) or the control group (linear incision technique with soft-tissue preservation) in a 1:1 ratio stratified for gender. Subjects at each site were randomised independent of other centres using randomisation software (Statistiska konsultgruppen, Gothenburg, Sweden). Blinding was not possible due to the type of intervention.

Procedures

ENT surgeons, experienced in the linear incision technique with soft-tissue preservation, performed all the surgeries and were given in-depth MIPS training before opening the trial for enrolment. The co-ordinating investigator and a surgical support team were present during the first few MIPS surgeries in the trial at every centre. Based on patient preference, local or general anaesthesia was used. Abutment length was determined by measuring the skin thickness prior to the administration of local anaesthetics. In both groups, a Ponto Wide 4-mm implant with a premounted abutment (9, 12 or 14 mm) was installed using an insertion torque setting of 40-50 Ncm (Oticon Medical, Askim, Sweden).

In the control group, the linear incision technique with soft-tissue preservation was performed (Figure 1A) (10,20). In the test group, the MIPS technique was performed according to the manufacturer's instructions (Figure 1B) (18-20). Detailed descriptions of the steps can be found in the protocol (20). Finally, in both groups, a healing cap was attached to the abutment and gauze drenched in antibiotic ointment (Terra-cortril, Pfizer Laboratories, New York, USA) was applied.

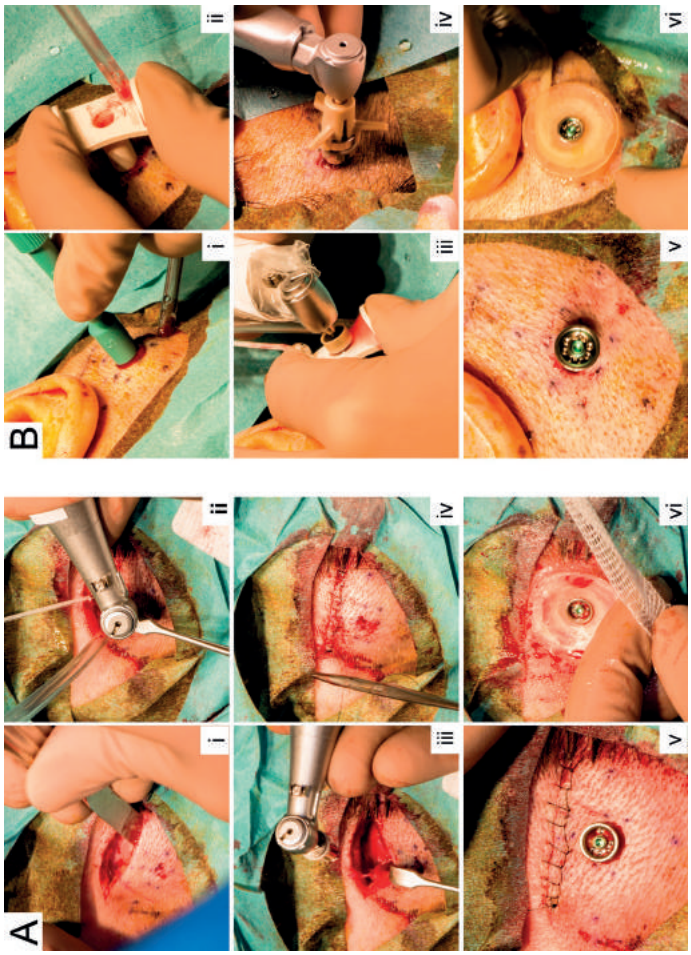

Figure 1 Surgical techniques: (A) Linear incision technique with soft-tissue preservation. (i) Linear incision. (ii) Drilling procedure. (iii) Implant installation. (iv) Closing incision line. (v) Result after skin punch. (vi) Attachment of healing cap and application of dressing. (B) Minimally Invasive Ponto Surgery (i) Skin punch. (ii) Placement of the cannula. (iii) Drilling procedure. (iv) Implant installation with installation indicator. (v) Result. (vi) Attachment of healing cap and application of dressing

Patients were assessed at inclusion, surgery, standard follow-up visits (9 days, 3 and 12 weeks) and extra consultations. The surgery procedure time (time from incision or punching to placement of healing cap) and total time in the operating theatre were measured. The peri-abutment skin was graded on all post-surgery visits according the Holgers Index (0 No irritation; 1 Slight redness; 2 Red and slightly moist tissue, no granuloma formation; 3 Reddish and moist; sometimes granulation tissue; 4 Removal of skin-penetrating implant necessary due to infection); (5) Skin reactions assessed as Holgers ≥ 2 were defined as an incidence of inflammation.

A detailed description of procedures and assessments is available in the published protocol (20) and supplementary data (S1). In short, pain scores, the presence of skin dehiscence and sagging, soft-tissue height, soft-tissue overgrowth and processor use were assessed on all follow-up visits, including extra consultations. Skin sensibility and wound healing were assessed on standard follow-up visits. Implant Stability Quotient (ISQ) (Osstell AB, Gothenburg, Sweden) measurements were obtained directly after surgery and on all follow-up visits. At the 12-week follow-up, cosmetic results and the skin pocket were assessed. In addition, complications, adverse events (AE), serious adverse events (SAE), device deficiencies and concomitant treatment were registered.

Outcomes

The outcomes have previously been described in detail (20). The primary end-point is the incidence of peri-abutment inflammation (Holgers Index ≥ 2) between surgery and the 12-week follow-up. Secondary outcomes include surgical procedure time, wound healing, the presence of dehiscence after surgery, soft-tissue overgrowth/height, loss of skin sensibility, pain, cosmetic results, ISQ measurements and extrusion rate. Tertiary outcomes include the skin pocket size and total time of processor use. Intra-operative complications, post-surgical complications, AEs, SAEs and device deficiencies were also noted.

Statistical analysis

Sixty-two subjects were needed to ensure sufficient power (20). For the primary end-point, a chi-square test was performed. Holgers Index scores on standard visits were compared using the Mantel-Haenzel chi-square test. In overall terms, continuous variables were compared using the Mann-Whitney U test. Dichotomous variables were compared using the chi-square test or Fisher's exact test in the event of low counts. A two-way analysis of variance was conducted on the influence of anaesthesia and surgical technique on the time spent in the operating theatre. A mixed model was used to analyse ISQ High and ISQ Low. The extrusion rate was compared using the log-rank test. Statistical significance was assumed at 0.05. All analyses were performed with an intention-to-treat (ITT) population and a per-protocol (PP) population.

Missing data were mainly handled using a last observation carried forward method. Sensitivity analyses were performed for the incidence of inflammation (Holgers Index)

and pain according to the following. For inflammation, the highest observed Holgers Index plus one was imputed in the sensitivity analysis, as well as a worst-case scenario using Holgers Index 4 scores. For the sensitivity analysis of pain scores, the highest possible value of 10 was imputed in a worst-case scenario.

Ethical considerations

This study was performed in accordance with ISO 14155:2011 and the Declaration of Helsinki (21). The study was approved by the ethics committee at Maastricht University Medical Centre+ (NL50072.068.14) and is registered with ClinicalTrials.gov NCT02438618. MCL and ZGT were added as sites after acceptance of the amendment to extend the study to a multicentre study. The local ethics committees approved the execution of the protocol at these sites. All subjects provided written informed consent.

This study is sponsored by Oticon Medical AB (Askim, Sweden). The investigators had full access to all data. Monitoring was performed by the sponsor and TFS Develop (Zaltbommel, The Netherlands). Data analysis was conducted by Statistiska Konsultgruppen (Gothenburg, Sweden).

Results

Patient demographics

Between December 2014 and August 2016, sixty-four subjects were included (Figure 2). Thirty-three subjects were randomised to the test group (52%) and thirty-one to the control group (48%). One subject was excluded during surgery due to the placement of a 3-mm implant, resulting in sixty-three subjects being analysed in the ITT population. For the PP population, five subjects who experienced an implant extrusion were excluded. The patient characteristics were similar between the groups (Table 1). The primary outcome, secondary outcomes and tertiary outcomes of the ITT population are presented in Table 2. The PP population results are presented in the supplementary data (S2-S3). Protocol deviations mainly included visits out of window.

Primary outcome

The incidence of inflammation (Holgers Index ≥ 2) between surgery and 12 weeks showed no statistically significant difference between surgical techniques in the ITT population ($p=0.37$) or PP population ($p=0.68$) (Figure 3A, Table 2 and Table S3). Five subjects experienced an episode of inflammation in the control group (16.7%) compared with three subjects in the test group (9.1%). Sensitivity analyses yielded similar results.

Secondary and tertiary end-points

Surgery

Surgery characteristics are presented in Table 1. Intra-operative events were few and comparable between the two groups (Table 2/S2). There were no conversions to linear incision for patients subjected to the MIPS surgery technique. The surgical procedure time was significantly shorter in the test group compared with the control group, with a mean time of 6.52 minutes (SD=2.84) and 13.3 minutes (SD=3.5) respectively (p<0.0001). The time spent in the operating theatre was significantly influenced by both the type of anaesthesia (p<0.0001) and the surgical technique (p=0.0062). Adverse events during surgery, device deficiencies and other device complaints are described in S5.

Table 1: Baseline and surgery characteristics

Baseline characteristics	MIPS(n=33)	Linear incision (n=30)
Age (years)	50.3 (16.3) (44.5; 56.1)	51.9 (16.1) (45.9; 57.9)
Gender		
Male	12 (36.4%)	11 (36.7%)
Female	21 (63.6%)	19 (63.3%)
Type of hearing loss		
Acquired conductive/mixed hearing loss	26 (78.8%)	25 (83.3%)
Single sided deafness	6 (18.2%)	5 (16.7%)
Congenital conductive hearing loss	1 (3.0%)	0 (0.0%)
Side scheduled for surgery		
Right	17 (51.5%)	13 (43.3%)
Left	16 (48.5%)	17 (56.7%)
Smoking		
No smoking	26 (78.8%)	22 (73.3%)
Smoking	7 (21.2%)	8 (26.7%)
Body Mass Index	27.4 (6.4) (25.2; 29.7)	28.3 (5.6) (26.2; 30.4)
Ethnicity		
Caucasian	33 (100.0%)	30 (100.0%)
Surgery characteristics	MIPS(n=33)	Linear incision (n=30)
Type of anaesthesia		
General	16 (48.5%)	17 (56.7%)
Local	17 (51.5%)	13 (43.3%)
Surgical time (minutes) *	6.52 (2.84) 6.00 (2.00; 15.00) (5.51; 7.52)	13.3 (3.5) 13.0 (8.0; 25.0) (12.0; 14.6)
Time in operation room (minutes)*/**	44.2 (11.1) 45.0 (28.0; 72.0) (40.1; 48.3)	50.8 (7.8) 50.5 (33.0; 69.0) (47.5; 54.1)
Skin thickness (millimetres)	6.12 (1.80) (5.48; 6.76)	6.03 (1.69) (5.40; 6.66)
Abutment length		
9	21 (63.6%)	13 (43.3%)

Baseline characteristics	MIPS(n=33)	Linear incision (n=30)
12	10 (30.3%)	16 (53.3%)
14	2 (6.1%)	1 (3.3%)
Manual tightening performed	12 (36.4%)	8 (26.7%)
Concomitant medication during surgery	28 (84.8%)	23 (76.7%)
Intra-operative events		
Drilling into vein	2 (6.1%)	2 (6.7%)
Dura mater exposed	0 (0.0%)	1 (3.3%)
Skin problems	0 (0.0%)	0 (0.0%)
Drilling into air pockets	0 (0.0%)	0 (0.0%)
Bleeding hematoma	1 (3.0%)	1 (3.3%)
Replacement suture	0 (0.0%)	0 (0.0%)

Categorical variables: n (%) is presented. Continuous variables: Mean (SD) (95% CI of Mean) is presented. *Median (Min; Max) (95% CI) is presented. ** Patients with additional intervention during surgery were excluded.

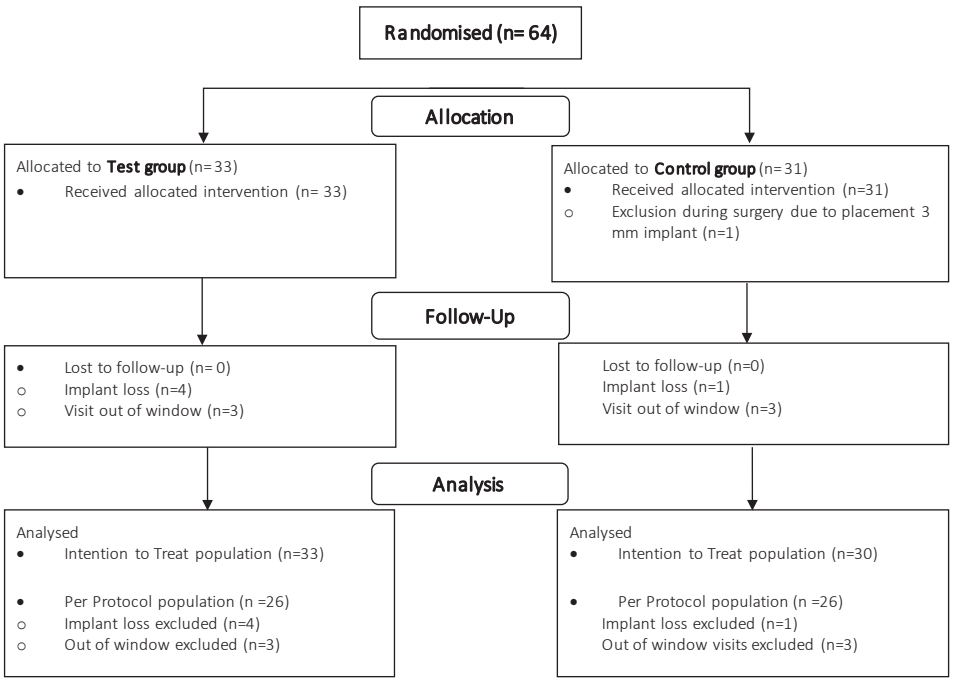

Figure 2: Subject flow chart Sixty-four subjects were randomised. Twenty-nine subjects in each group were included in the per protocol analysis

Soft-tissue outcomes

Wound healing at the implant-soft-tissue interface did not differ significantly between groups (Table 2). All wounds had healed after 12 weeks. At the nine-day follow-up, a slight dehiscence of the skin-abutment interface was observed in 14 subjects (48.5%) in the test

group compared with 21 subjects (73.3%) in the control group (p=0.078). An additional analysis of the Holgers Index ratings per visit revealed no difference at nine days or 12 weeks (p=0.33, p=0.64) (Figure 3A). At three weeks, a significantly larger number of cases with Holgers 1 scores was observed in the test group compared with the control group (40.6% vs 10%, p=0.027). Skin sagging, mainly observed in the most cranial posterior quadrant, was present in eight subjects (27.6%) and 20 subjects (71.4%) in the test and control group respectively (p=0.002). There were no cases of soft-tissue overgrowth or significant differences in skin height between treatment groups.

Sensibility and pain

Loss of sensibility was significantly less in the test group compared with the control group on all follow-up visits (Table 2, Figure 3B). At nine days, the mean loss of sensibility was 2.70 mm (SD=6.13) and 13.5 mm (SD=21.0) for the test and control group respectively (p=0.005, Table 2, Figure 3B). At 12 weeks, the maximum area affected was 2 mm in the test group and 60 mm in the control group. No significant differences in pain scores for pain around the BAHS, radiating pain or headache related to the BAHS were observed (Table 2).

Cosmetic results

Natural skin position, extent of baldness, scarring, skin colour and indentation, as well as overall observer scores, were all significantly better in the test group compared with the control group (Table 2, Figure 3C). Subject satisfaction scores relating to cosmetic results, with or without the processor attached, did not differ significantly between groups.

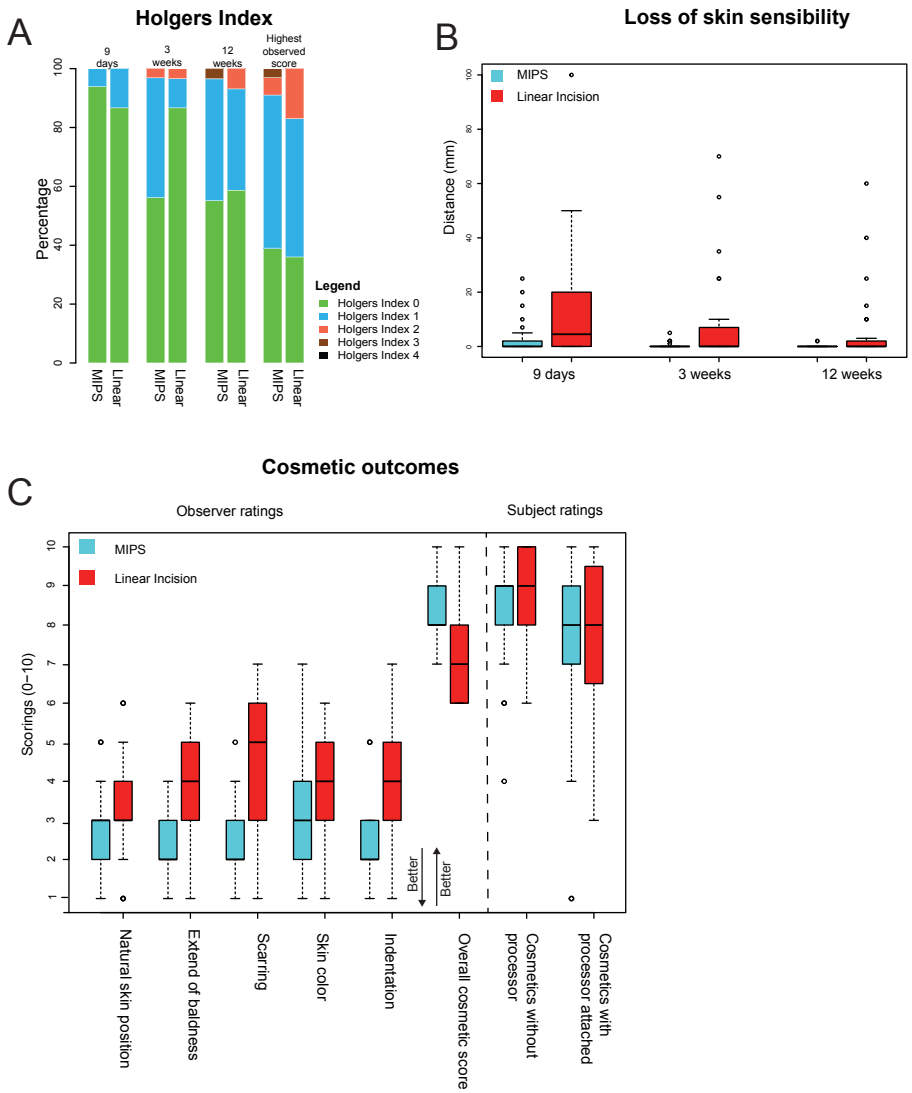

Figure 3: Primary outcome, loss of skin sensibility and cosmetic aspects (A) Stacked bar chart for the Holgers Index scores on standard follow-up visits and the highest observed Holgers Index score. (B) Box plots of loss of skin sensibility per treatment group on standard follow-up visits. (C) Cosmetic outcomes at 12 weeks per treatment group. Cosmetic outcome specifics are described in Table 2.

Table 2 Outcomes

Primary outcome	MIPS (n=33)	Linear incision (n=30)	p-value
Holgers index >= 2 at any time from surgery to 12 weeks	3 (9.1%)	5 (16.7%)	0.37
Holgers index >= 2 at any time from surgery to 12 weeks (Sensitivity analysis, highest observed Holgers Index score plus one)	3 (9.1%)	5 (16.7%)	0.37
Holgers index >= 2 at any time from surgery to 12 weeks (Sensitivity analysis, all implant losses have experienced Holgers Index score of 4)	7 (21.2%)	5 (16.7%)	0.65

Secondary outcomes	MIPS (n=33)	Linear incision (n= 30)	p-value
Wound dehiscence at 9 days	16 (48.5%)	22 (73.3%)	0.078
Extrusion rate	4 (12.1%)	1 (3.3%)	0.19
Loss of skin sensibility (mm)*			
9 days	2.70 (6.13) 0.00 (0.0; 25.0) (0.52; 4.87)	13.5 (21.0) 4.5 (0.0; 100.0) (5.6; 21.3)	0.0050
3 weeks	0.375 (1.04) 0.0 (0.0; 5.0) (0.00; 0.75)	8.23 (17.25) 0.0 (0.0; 70.0) (1.79; 14.68)	0.013
12 weeks	0.14 (0.52) 0.0 (0.0; 2.0) (0; 0.33)	5.79 (13.75) 0.00 (0.00; 60.00) (0.56; 11.02)	0.0076
No loss of sensibility (0 mm)			
9 days	24 (72.7%)	13 (43.3%)	
3 weeks	27 (84.4%)	18 (60.0%)	
12 weeks	27 (93.1%)	19 (65.5%)	
Soft tissue overgrowth	0 (0.0%)	0 (0.0%)	1.00
Mean skin level at 12 weeks	5.02 (1.42) (4.48; 5.56)	5.08 (1.04) (4.68; 5.47)	0.64
Wound healing			
9 days	7 (21.2%)	5 (16.7%)	0.89
3 weeks	22 (68.8%)	20 (71.4%)	0.82
12 weeks	29 (100.0%)	29 (100.0%)	1.00
Pain scorings*			
Pain around implant			
9 days	1.39 (1.87) 0.00 (0.00;6.00) (0.73; 2.06)	1.97 (2.61) 1.00 (0.00:8.00) (0.99; 2.94)	0.50
3 weeks	0.938 (1.22) 0.0 (0.00;4.00) (0.50; 1.38)	1.0 (1.61) 0.0 (0.00;6.00) (0.04; 1.60)	0.67
12 weeks	1.38 (2.23) 0.00 (0.00;8.00) (0.53; 2.23)	1.17 (2.04) 0.00 (0.00;7.00) (0.40; 1.95)	0.54
Radiating pain			
9 days	0.61 (1.66) 0.0 (0.00;7.00) (0.02; 1.19)	0.5 (1.57) 0.0 (0.00;8.00) (0.0; 1.09)	0.95
3 weeks	0.56 (1.39) 0.0 (0.00;5.00) (0.06; 1.06)	0.43 (1.36) 0.0 (0.00;5.00) (0.0; 0.94)	0.39
12 weeks	0.76 (1.86) 0.0 (0.00;6.00) (0.05; 1.47)	0.76 (1.8) 0.0 (0.00;7.00) (0.04; 1.48)	0.77

Secondary outcomes	MIPS (n=33)	Linear incision (n= 30)	p-value
Presence of headache			
9 days	0.42 (1.39) 0.0 (0.00;7.00) (0.0; 0.92)	1.30 (2.39) 0.00 (0.00;8.00) (0.41; 2.19)	0.077
3 weeks	0.375 (1.476) 0.0 (0.00;6.00) (0.0; 0.91)	0.30(1.32) 0.0 (0.00;7.00) (0.0; 0.79)	0.96
12 weeks	0.79 (2.1) 0.0 (0.00;8.00) (0.00; 1.59)	0.24 (0.83) 0.0 (0.00;4.00) (0.08; 0.56)	0.59
Skin sagging at 12 weeks			
Quadrant 1	4 (13.8%)	4 (14.3%)	1.00
Quadrant 2	7 (24.1%)	19 (67.9%)	0.0020
Quadrant 3	1 (3.4%)	1 (3.6%)	1.00
Quadrant 4	1 (3.4%)	2 (7.1%)	0.97
Any quadrant	8 (27.6%)	20 (71.4%)	0.0020
Cosmetic results^			
Observer scorings			
Natural skin position	2.72 (1.10) (2.31; 3.14)	3.48 (1.38) (2.96; 4.01)	0.025
Extent of baldness	2.24 (0.79) (1.94; 2.54)	3.62 (1.35) (3.11; 4.13)	<.0001
Scarring	2.41 (0.95) (2.05; 2.77)	4.48 (1.79) (3.80; 5.16)	<.0001
Skin colour	3.17 (1.23) (2.71; 3.64)	3.86 (1.27) (3.38; 4.35)	0.020
Indentation	2.34 (1.01) (1.96; 2.73)	4.00 (1.63) (3.38; 4.62	<.0001
Overall cosmetic score	8.45 (0.74) (8.17; 8.73)	7.17 (1.20) (6.72; 7.63)	<.0001
Subject scorings			
Without processor (BAHS)	8.42 (1.47) (7.83; 9.02)	8.61 (1.29) (8.11; 9.11)	0.75
With processor attached	7.41 (2.58) (6.39; 8.43)	7.89 (1.83) (7.18; 8.60)	0.73
Tertiary outcome	MIPS (n=29)	Linear incision (n=29)	
Pocket size (normal position) (mm)	0.207 (0.292) (0.096; 0.318)	0.172 (0.251) (0.077; 0.268)	NP
Pocket size (maximum) (mm)	0.672 (0.418) (0.513; 0.831)	0.698 (0.440) (0.531; 0.866)	NP
Sound processor usage (hours per week)	70.5 (37.2) (56.3; 84.6)	90.3 (25.5) (80.3; 100.4)	NP

Categorical variables: n (%). Continuous variables: Mean (SD) (95% CI). * Mean (SD), Median (Min; Max) (95% CI of the mean). NP = not planned. ^Cosmetic rating: Observer outcomes (not including overall cosmetic score): 1-10. 1 being no difference with the healthy contra-lateral site, with 10 being the most negative difference with the healthy situation. Overall cosmetic and subject scorings: 1-10: 10 being the best cosmetic result and 1 being the most negative cosmetic result.

Implant loss

During the 12-week follow-up period, four implants in the test group were extruded (12.1%) compared with one in the control group (3.3%) (Figure 4A). Implant loss occurred between 25 days and 90 days post-surgery. A non-significant p-value of 0.19 was found using the log-rank test (hazard ratio = 3.89, 95% CI = 0.4; 34.8).

Implant stability quotient

ISQ High and Low on standard visits are presented in Figure 4B. Both ISQ High and ISQ Low were significantly influenced by the surgical treatment (p=0.014, p=0.007) and abutment length (p<0.001, p<0.001). In overall terms, ISQ High was influenced by time (p<0.002), but ISQ Low was not (p=0.38). The model is presented in S4. In the test group, ISQ High was 2.35 points (95% CI = -4.21, - 0.49) lower and ISQ Low was 2.7 points lower (95% CI = -4.65, -0.76) compared with the control group. No obvious association was observed between the initial ISQ and implant loss (S6).

Serious adverse events, adverse events and device complaints

Serious adverse events, adverse events, device complaints and device deficiencies are presented in the supplementary data (S5). Common and expected complications related to BAHS were observed in both groups. Thirty-one subjects (91.4%) in the test group and 25 subjects (83.3%) in the control group had at least one reported AE, with a total of 168 AEs.

Discussion

Principal findings

This randomised, controlled, clinical trial compared the outcomes between surgery and the 12 week follow-up of two surgical procedures for the installation of BAHS: a new minimally invasive surgical technique (test) and the linear incision technique with soft-tissue preservation (control). No significant differences in the incidence of inflammation (Holgers ≥ 2) were found between procedures. However, MIPS surgery resulted in a significantly better outcome in terms of sensibility, surgical time, time spent in the operating theatre and cosmetic results. In addition, significantly less skin sagging and a tendency towards less dehiscence were observed in the test group. The test group exhibited significantly lower ISQ values and the extrusion rate was non-significantly higher.

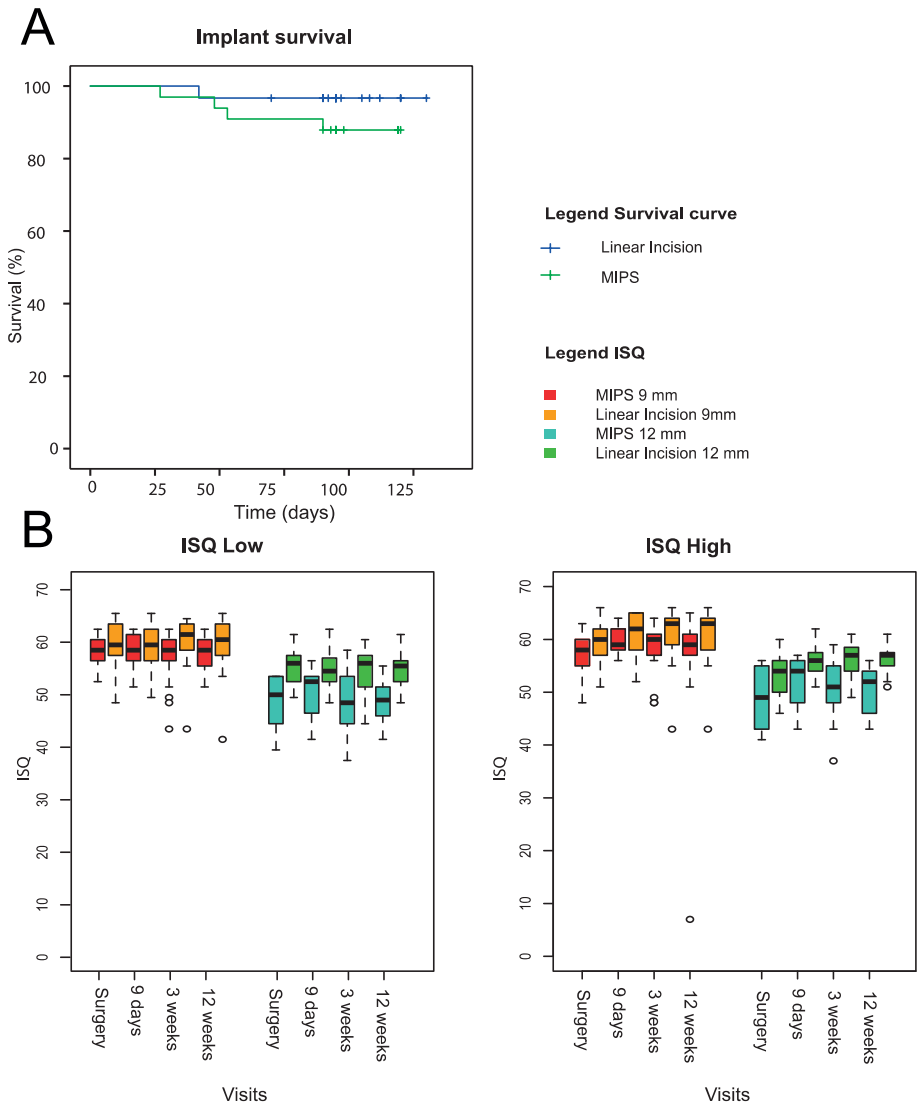

Figure 4: Extrusion rate and ISQ values (A) Kaplan-Meier survival plot for bone conduction hearing implants displayed per surgical technique. (B) Boxplots of ISQ measurements at surgery and on standard follow-up visits. ISQ measurements are displayed for ISQ Low and ISQ High per abutment length and surgical technique.

Surgery

Few intra-operative events or adverse events were observed, with no clear differences between surgical techniques, thereby underscoring the reliability of both the linear incision technique and the punch-only approach. The minimally invasive nature of the MIPS procedure, the reduced surgical time, together with an efficient drilling sequence, make it a suitable technique to be performed under local anaesthesia. Several surgeons have indicated that MIPS appears deceptively easy and that there is a learning curve (19). Similar experiences were observed in dental surgery when the flapless placement of dental implant systems was introduced. There is a need for appropriate training, as there appears to be a learning curve to achieve treatment success (22,23).

Soft-tissue outcomes

Although no difference in the incidence of inflammation was observed between groups, on the three-week follow-up visit, more mild skin reactions (Holgers = 1) were observed in the test group. In addition, for both techniques, a slight dehiscence with non-epithelialised skin was often observed at nine days and it had typically disappeared by three weeks. It is possible that the observed mild skin reaction at three weeks in the MIPS group reflects a difference in the temporal course of the healing process compared with the linear incision technique where, in contrast to MIPS, a flap is raised. Moreover, we believe that skin sagging could be influenced by the surgical manipulation of the skin and positioning during surgery. The fact that skin sagging was less prevalent using the MIPS technique corroborates this hypothesis. Despite these differences in soft-tissue outcomes, wound healing was comparable between techniques in the first three months. The long-term data for these patients will be published when available. To acquire a greater understanding of the mechanisms underpinning healing and soft-tissue reactions, additional tools such as quantitative polymerase chain reaction (qPCR) and microbiota were applied in this study and will supplement the findings reported here. It is to be hoped that this will enable an understanding of the temporal course of the tissue response.

Other outcomes

The presence and the extent of loss of sensibility and cosmetic appearance have historically been an under-reported adverse patient outcome after BAHS implantation using the tissue reduction technique. Recent studies with a tissue preservation approach have shown improvements (9,10). In this study, numbness was even less prevalent after MIPS surgery compared with a linear incision approach. As a result, this could now be regarded as irrelevant in relation to MIPS. Cosmetic scoring results with the processor mounted on the BAHS were lower compared with the results without a mounted processor. As demonstrated in our results, it could be advantageous to use a scoring system that includes cosmetic results with and without the processor.

Adverse events

In this study, we meticulously gathered possible AEs and this is the most likely explanation for the relatively large number of observed AEs. In overall terms, we found no clear differences in AEs between techniques. Difficulty sleeping on the implant side of the head was frequently spontaneously mentioned. This has previously never been described. To facilitate skin preservation techniques, abutment lengths have increased. The abutment Inadvertently sticks out further from the skull, which may explain this complaint. The severity of this complaint needs to be evaluated before it can be compared or put in context with other complaints such as pain, loss of skin sensibility or inflammation.

ISQ

The mixed model revealed an association between the ISQ and abutment length, as well as the time after surgery. This is in line with previous findings (9,24–26). Interestingly, the ISQ was significantly influenced by the surgical technique. Both ISQ High and Low were approximately 2.5 points lower in the test group compared with the control group. In comparison, abutment length influenced ISQ values by 6-12 points. Associations between the ISQ and surgical technique have previously not been reported for BAHS, although an effect of this kind has been reported for dental implants (27). Compared with the effect the abutment length has on ISQ values, the difference in the ISQ between the test and control group is small and the clinical relevance is probably insignificant. Furthermore, we found no relationship between the primary ISQ values and the extrusion rate (S6).

Extrusion

Studies published in recent years report high implant survival rates, even when using punch-only techniques, with loss rates between 0% and 5.8% being reported (9,15,17,25). We observed a survival rate of 88% for MIPS. During the last few decades, BAHS surgical tools have undergone only minor adjustments. For MIPS, a new drilling protocol with guided drilling via the cannula was developed to facilitate a flapless approach. These drills are more efficient and require less manual pressure, resulting in different tactile feedback to the surgeon compared with the classical systems (28). Several explanations have been postulated throughout the introduction of flapless approaches for dental implants and they could also be applicable to MIPS (29,30). Diminished visibility may lead to an angulated drilling/implant placement or incomplete insertion. Reduced access for external irrigation may lead to thermal damage (31,32). An *in-vitro* study comparing heat generation for flap and flapless drilling showed that the temperature was slightly higher for the flapless procedure in dental implants (33). Extrusion is a concern associated with MIPS and a possible association with cooling and implant positioning requires further study. Training, following instructions and caution all appear to be relevant factors for success.

Strengths and limitations

One of the main strengths of this study is the multicentre, randomised, controlled design, with a large sample size of 63 subjects. In this study, we re-evaluated outcome measurements to increase reliability. In addition, adverse events were gathered in conjunction with regular follow-up visits and extra consultations. We believe that the set-up of this study allows for a better estimation of complications such as inflammation due to the stringency applied to calculate and use a cumulative percentage of all visits, including extra visits. To allow for a correct comparison, no differences in implant type were allowed. Several limitations are relevant to this study. Healing, dehiscence and the Holgers Index possibly influence each other, warranting some caution when interpreting the observed soft-tissue outcomes. A standardised, well-defined outcome measurement set would improve BAHS-related outcomes. The recently established AuroNet could aid in the creation of a standardised outcome set of this kind (34). Although all the surgeons were trained prior to the first MIPS surgery in the trial, experience between techniques differed. As this technique and instruments are different, a learning curve effect could also play a role. In our study population, due to chance all subjects were of Caucasian origin, limiting the general applicability of this data to a more mixed ethnic situation. Scar formation and BAHS related skin complications have been shown to be affected by ethnicity (35). The previously observed higher rate of complications in African Americans (35) might even benefit more from improved outcomes.

Perspective

Encouraging outcomes, particularly patient-centred outcomes such as sensibility loss and cosmetic appearance, were observed for MIPS compared with the linear incision with soft-tissue preservation. Despite the fact that a non-significant statistical difference was found, extrusion remains a concern related to MIPS. Although reduced cooling might be an aggravating factor, an assumption like this requires careful evaluation and follow-up.

Longer term 22-month follow-up results from this study are expected to become available in the second half of 2018. Therein, data will be provided on the incidence of soft tissue inflammation, long term processor usage and changes in quality of life over time within subjects. At this point in time, a well-founded recommendation on surgical technique can hopefully be made.

As presented in our results, the relationship between the ISQ and extrusion is not straightforward, warranting further clinical data on an association, or lack thereof, between the ISQ and biomechanical stability. Both the inter- and intra-rater reliability of the Holgers Index and biological validity would benefit from further study. As part of this clinical study, samples were taken for bacteria and tissue status to further investigate a subset of research questions (20). These data will hopefully shed further light on the correlations (or lack thereof) between clinical parameters, the Holgers index, the ISQ and the biological tissue responses.

Conclusion and recommendation

No significant differences between MIPS and the linear incision technique were observed in terms of skin inflammation in the first three months. MIPS results in a statistically significant reduction in the loss of skin sensibility, less skin sagging, improved cosmetic results and reduced surgical time. Although non-significant, the implant extrusion rate warrants further research.

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Supplementary material

S1: Schedule of endpoint assessments Follow-up visits are planned post-surgery. Pain assessment: Score for pain around the implant / radiating pain / headache associated with implant. Cosmetic result scores: Score for natural skin position, baldness, scarring, skin color, indentation, overall cosmetic score. Figure adapted with permission from Calon *et al.* (21)

Outcomes	Description	Units	Inclusion	Surgery	9 Days	3 Weeks	12 weeks	Extra consultation
Inclusion								
Informed consent			X					
Baseline characteristics			X					
Primary outcome								
Incidence of inflammation	Scoring according to the Holgers Index.	0-4			x	x	x	x
Secondary outcomes								
Surgical procedure time	Time during surgery and time in operation theater.	Minutes		x				
Wound healing time	Time to initial healing.	Yes/No/ Partial			x	x	x	
RFA: ISQ measurements	Horizontal and vertical ISQ values	0-100		x	x	x	x	x
Pain	Pain assessment	0-10			x	x	x	x
Soft tissue height/ overgrowth	Distance from abutment to skin in four quadrants.	Millimeters			x	x	x	x
Dehiscence	Presence of dehiscence.	Yes/No			x	x	x	x
Skin sagging	Presence of skin sagging in four quadrants.	Yes/No			x	x	x	x
Extrusion	Implant extrusion at any time point.	Yes/No			x	x	x	x
Loss of skin sensibility	Loss of sensibility from abutment to the most outer point.	Millimeters			x	x	x	
Cosmetic results	Cosmetic result scores.	1-10					x	

S2 Baseline and surgery characteristics pp population

Baseline characteristics	MIPS (n=26)	Linear incision (n=26)
Age (years)	51.2 (15.0) (45.2; 57.2)	52.4 (15.9) (46.0; 58.8)
Gender		
Male	8 (31%)	11 (42%)
Female	18 (69%)	15 (58%)
Type of hearing loss		
Acquired conductive/mixed hearing loss	19 (73%)	22 (85%)
Single sided deafness	6 (23.0%)	4 (15%)
Congenital conductive hearing loss	1 (4%)	0 (0%)
Side scheduled for surgery		
Right	14 (54%)	11 (42%)
Left	12 (46%)	15 (58%)
Smoking		
No smoking	21 (81%)	20 (77%)
Smoking	5 (19%)	6 (23%)
Body Mass Index	27.9 (6.6) (25.2; 30.5)	28.8 (5.8) (26.5; 31.1)
Ethnicity		
Caucasian	26 (100%)	26 (100%)
Surgery characteristics	MIPS (n=26)	Linear incision (n=26)
Type of anaesthesia		
General	12 (46%)	13 (50%)
Local	14 (54%)	13 (50%)
Surgical time (minutes) *	6.7 (2.9) 6.0 (2.0; 15) (5.5; 7.9)	13.1 (3.4) 13 (9; 25) (11.8; 14.5)
Time in operation room (minutes)*/**	43.2 (11.2) 42.5 (28; 72) (38.5; 48)	49.5 (6.7) 50 (33;61) (46.3; 52.6)
Skin thickness (millimetres)	6.2 (1.9) (5.5; 7.0)	6.2 (1.7) (5.5; 6.8)
Abutment length		
9	17 (65%)	11 (42%)
12	7 (27%)	14 (54%)
14	2 (8%)	1 (4%)
Manual tightening performed	11 (42%)	8 (31%)
Concomitant medication during surgery	21 (81%)	20 (77%)
Intra-operative events		
Drilling into vein	2 (8%)	1 (4%)
Dura mater exposed	0 (0.0%)	0 (0%)
Skin problems	0 (0.0%)	0 (0%)
Drilling into air pockets	0 (0.0%)	0 (0%)
Bleeding hematoma	1 (4%)	1 (4%)
Replacement suture	0 (0.0%)	0 (0.0%)

Categorical variables: n (%). Continuous variables: Mean (SD) (95% CI of Mean). *Median (Min; Max) (95% CI).
** Patients with additional intervention during surgery were excluded.

S3: Primary and secondary outcomes PP population

Outcomes			
Primary outcome	MIPS (n=26)	Linear incision (n=26)	p-value
Holgers index >= 2 at any time from surgery to 12 weeks	3 (12%)	4 (15%)	0.68
Secondary outcomes	MIPS (n=26)	Linear incision (n= 26)	p-value
Wound dehiscence at 9 days	13 (59%)	20 (77%)	0.08
Loss of skin sensibility *			
9 days	3.2 (6.8) 0 (0;25) (0.4; 5.9)	15.3 (22) 10 (0;100) (6.4; 24.2)	<0.01
3 weeks	0.4 (1.1) 0 (0; 5) (0;0.8)	9.1 (18.4) 0 (0; 70) (1.7; 16.5)	0.03
12 weeks	0.2 (0.5) 0 (0; 2) (0;0.4)	5 .8 (14.4) (0) (0; 60) (0; 11.6)	0.03
No loss of sensibility (0 mm)			
9 days	19(73%)	11 (42%)	<0.05
3 weeks	22 (85%)	16 (62%)	0.12
12 weeks	24 (92%)	18 (69%)	0.08
Soft tissue overgrowth	0 (0%)	0 (0%)	1.00
Mean skin level at 12 weeks	5.1 (1.5) 5 (4.5; 5.6)	5.0 (0.9) 5 (4.9; 5.6)	0.41
Wound healing			
9 days	4 (15%)	3 (12%)	1.0
3 weeks	17 (65%)	17 (68%)	1.0
12 weeks	26 (100%)	26 (100%)	1.0
Pain scorings*			
Pain around implant			
9 days	1.6 (2) 1 (0;6) (0.8;2.4)	2.2 (2.7) 1 (0;7) (1.1; 3.3)	0.60
3 weeks	1.0 (1.2) 0.5 (0;4) (0.5;1.5)	0.8 (1.4) 0 (0;5) (0.3;1.4)	0.45
12 weeks	1.5 (2.3 0 (0;8) (0.6;2.5)	1.1 (2.1) 0 (0; 7) (0.3; 2.0)	0.26
Radiating pain			
9 days	0.8 (1.8) 0 (0;7) (0; 1.5)	0.5 (1.7) 0 (0; 8) (0; 1.2)	0.69
3 weeks	0.7 (1.5) 0 (0; 5) (0.1; 1.3)	0.3 (1.1) 0 (0, 5) (0; 0.8)	0.15
12 weeks	0.8 (2) 0 (0; 6) (0.1; 1.6)	0.8 (2) 0 (0; 7) (0; 1.6)	0.55
Presence of headache			
9 days	0.5 (1.6) 0 (0; 7) (0; 1.2)	1.2 (2.4) 0 (0; 8) (0.3 2.2)	0.28
3 weeks	0.5 (1.6) 0 (0;6) (0; 1.1)	0.1 (0.4) (0; 2) (0; 0.2)	0.54
12 weeks	0.9 (2.2) 0 (0;8) (0; 1.8)	0.3 (0.9) 0 (0; 4) (0; 0.6)	0.58
Skin sagging at 12 weeks			
Quadrant 1	3 (12%)	4 (15%)	1.0
Quadrant 2	6 (23%)	19 (73%)	<0.001
Quadrant 3	1 (4%)	0 (0 %)	1.0
Quadrant 4	1 (4%)	2 (8%)	1.0
Any quadrant	7 (27%)	20 (77%)	<0.001
Cosmetic results^			
Observer scorings			
Natural skin position	2.7 (1.1) (2.2; 3.1)	3.7 (1.3) (3.1; 4.2)	<0.001

Secondary outcomes	MIPS (n=26)	Linear incision (n= 26)	p-value
Extent of baldness	2.2 (0.8) (1.9; 2.5)	3.8 (1.3) (3.3; 4.3)	p<0.001
Scarring	2.4 (1) (2; 2.8)	4.7 (1.7) (4; 5.4)	p<0.001
Skin colour	3.2 (1.3) (2.7; 3.7)	4 (1.1) (3.5; 4.5)	0.02
Indentation	2.3 (1) (1.9; 2.7)	4.2 (1.6) (3.6; 4.8)	p<0.001
Overall cosmetic score	8.4 (0.8) (8.1; 8.7)	7.0 (1.1) (6.6; 7.4)	p<0.001
Subject scorings			
Without processor (BAHS)	8.5 (1.5) (7.9; 9.2)	8.5 (1.3) (8; 9.1)	0.85
With processor attached	7.2 (2.6) (6.1; 8.3)	7.7 (1.9) (7; 8.5)	0.69

Categorical variables: n (%). Continuous variables: Mean (SD) (95% CI). * Mean (SD), Median (Min; Max) (95% CI of the mean). ^Cosmetic rating: Observer outcomes (not including overall cosmetic score): 1-10. 1 being no difference with the healthy contra-lateral site, with 10 being the most negative difference with the healthy situation. Overall cosmetic and subject scorings: 1-10: 10 being the best cosmetic result and 1 being the most negative cosmetic result.

S4 Mixed model results ISQ

	ISQ High				ISQ Low			
	B	95% confidence interval		*p*	*B*	*95% confidence interval*		*p*
Intercept	59.12	57.34	60.91	<0.001	59,93	58,11	61,74	<0.001
Linear Incision	-	-	-	-	-	-	-	-
MIPS	-2.35	-4.21	-0.49	0.014	-2,70	-4,65	-0,76	0.007
9 mm abutment	-	-	-		-	-	-	-
12 mm abutment	-6.06	-7.97	-4.14	<0.001	-6,57	-8,57	-4,57	<0.001
14 mm abutment	-11.53	-15.86	-7.21	<0.001	-11,89	-16,42	-7,35	<0.001
Surgery	-	-	-	-	-	-	-	-
9 days	2.43	1.65	3.21	<0.001	0.22	-0.50	0.94	0.54
3 weeks	1.72	0.73	2.71	0.001	-0.56	-1.58	0.45	0.27
12 weeks	0.99	-1.24	3.23	0.377	-1.18	-3.48	1.13	0.31

Results for Mixed model. Differences compared to the linear incision technique (technique), 9 mm abutment (abutment length) and surgery (timings) are described. For ISQ High, overall timings influenced ISQ High. For ISQ Low, timing did not influence ISQ.– indicates reference variable

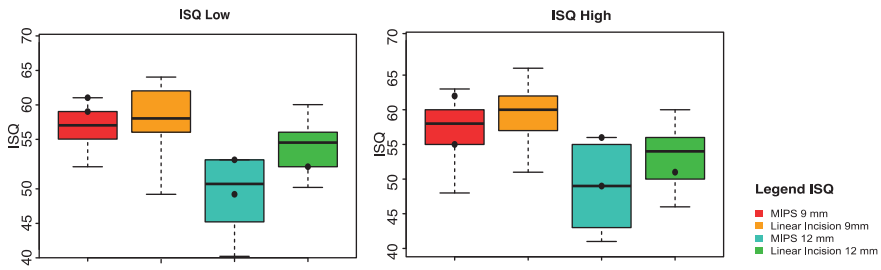
S5: Serious adverse events, Adverse events, device complaints and device deficiencies All AEs are presented according MEDRA coding.

	Total (n=63)		Test group (MIPS) (n=33)		Control group (linear incision) (n=30)	
	AEs	Subjects with AEs n (%)	AEs	Subjects with AEs n (%)	AEs	Subjects with AEs n (%)
Serious adverse events*						
Cardiac disorders						
Atrial fibrillation	1	1 (1.6)	1	1(3.0%)	0	0(0)
General disorders and administration conditions						
Device expulsion (implant extrusion) *	5	5 (7.9%)	4	4(12.1%)	1	1(3.3%)
Device deficiencies						
Device difficult to use	1	1(1.6%)	1	1(3.0%)	0	0(0)
Device deployment issue	2	2(3.2%)	1	1(3.0%)	1	1(3.3%)
Device issue (Abutment inserter)	2	2(3.2%)	2	2(6.1%)	0	
Device issue (Healing cap)	11	11(17.5%)	6	6(18%)	5	5(16.7%)
Device connection issue (Sound processor)	4	4(6.3%)	2	2(6.1%)	2	2(6.7%)
Device malfunction (Sound processor)	3	3(6.3%)	1	1(3.0%)	2	2(16.7%)
Device malfunction (Air conduction hearing aid)	1	(1.6%)	0	0(0)	1	1(3.3%)
Device complaints & issues						
Rescheduled surgery due to patient compliance/logistics	2	2(3.2%)	1	1(3.0%)	1	1(3.3%)
Dropped surgical tool leading prolonged surgery	1	1(1.6%)	0	0(0)	1	1(3.3%)
Implant placement without visual feedback	1	1 (1.6%)	1	1(3.0%)	0	0(0)
Difficulty to estimate bone thickness through cannula	1	1(1.6%)	1	1(3.0%)	0	0(0)
Sound from abutment on pillow	1	1(1.6%)	0	0(0)	1	1(3.3%)
Adverse events (AE)						
Any AE	168	56(88.8%)	89	31(93.4%)	80	25(83.3%)
Cardiac disorders						
Cardiac disorder	3	3(4.8%)	3	3(9.1%)	0	0(0)
Ear and labyrinth disorders						
Ear pain	1	1(1.6%)	1	1(3.0%)	0	0(0)
Eye disorders						
Vision blurred	1	1(1.6%)	0	0(0)	1	1(3.3%)
Gastrointestinal disorders						

Adverse events (AE)						
General disorders and administration site condition						
Abdominal pain upper	1	1(1.6%)	0	0(0)	1	1(3.3%)
Application site haemorrhage	2	2(3.2%)	2	2(6.1%)	0	0(0)
Application site pain	0	0(0)	0	0(0)	1	1(3.3%)
Fatigue	2	2(3.2%)	2	2(6.1%)	0	0(0)
Implant site erythema	7	7(11.1%)	5	5(15.2%)	2	2(6.7%)
Implant site reaction	1	1(1.6%)	0	0(0)	1	1(3.3%)
Impaired healing	3	3(4.8%)	1	1(3.0%)	2	2(6.7%)
Inflammation	11	11(17.5%)	4	4(12.1%)	7	7(23.3%)
Injury associated with device	1	1(1.6%)	1	1(3.0%)	0	0(0)
Pain	12	11(17.5%)	7	6(18.2%)	5	5(16.7%)
Immune system disorders						
Immunodeficiency common variable	1	1(1.6%)	1	1(3.0%)	0	0(0)
Infections and infestations						
Furuncle	1	1(1.6%)	0	0(0)	1	1(3.3%)
Influenza	6	5(7.9%)	2	1(3.0%)	4	4(13.3%)
Otitis externa	2	2(3.2%)	0	0(0)	2	2(6.7%)
Otitis media	2	2(3.2%)	2	2(6.1%)	0	0(0)
Wound dehiscence	43	43(68.3%)	20	20(60.6%)	23	23(76.7%)
Injury, poisoning and procedural complications						
Anaesthetic complication	5	5(7.9%)	2	2(6.1%)	3	3(10.0)
Eschar (crust formation)	18	17(27.0%)	10	10(30.3%)	8	7(23.3%)
Inadequate osteointegration	6	6(9.5%)	4	4(12.1%)	2	2(6.7%)
Post procedural haemorrhage	1	1(1.6%)	1	1(3.0%)	0	0(0)
Procedural nausea	3	3(4.8%)	3	3(9.1%)	0	0(0)
Metabolism and nutrition disorders						
Hypoglycaemia	1	1(1.6%)	0	0(0)	1	1(3.3%)
Musculoskeletal and connective tissue disorders						
Arthralgia	1	1(1.6%)	1	1(3.0%)	0	0(0)
Nervous system disorders						
Dizziness	6	6(9.5%)	3	3(9.1%)	3	3(10%)
Hypaesthesia	3	3(4.8%)	0	0(0)	3	3(10%)
Paraesthesia	1	1(1.6%)	0	0(0)	1	1(3.3%)
Psychiatric disorders						
Insomnia	7	7(11.1%)	6	6(18.2%)	1	1(3.3%)
Respiratory, thoracic and mediastinal disorders						
Dyspnoea	2	1(1.6%)	2	1(3.0%)	0	0(0)
Skin and subcutaneous tissue disorders						

Adverse events (AE)						
Ingrown hair	1	1(1.6%)	0	0(0)	1	1(3.3%)
Pruritus	5	5(7.9%)	2	2(6.1%)	3	3(10%)
Skin atrophy	1	1(1.6%)	0	0(0)	1	1(3.3%)
Skin exfoliation	1	1(1.6%)	0	0(0)	1	1(3.3%)
Skin irritation	5	5(7.9%)	3	3(9.1%)	2	2(6.7%)
Skin hypertrophy	1	1(1.6%)	1	1(3.0%)		
Vascular disorders						
Haemorrhage	2	2(3.2%)	1	1(3.0%)	1	1(3.3%)

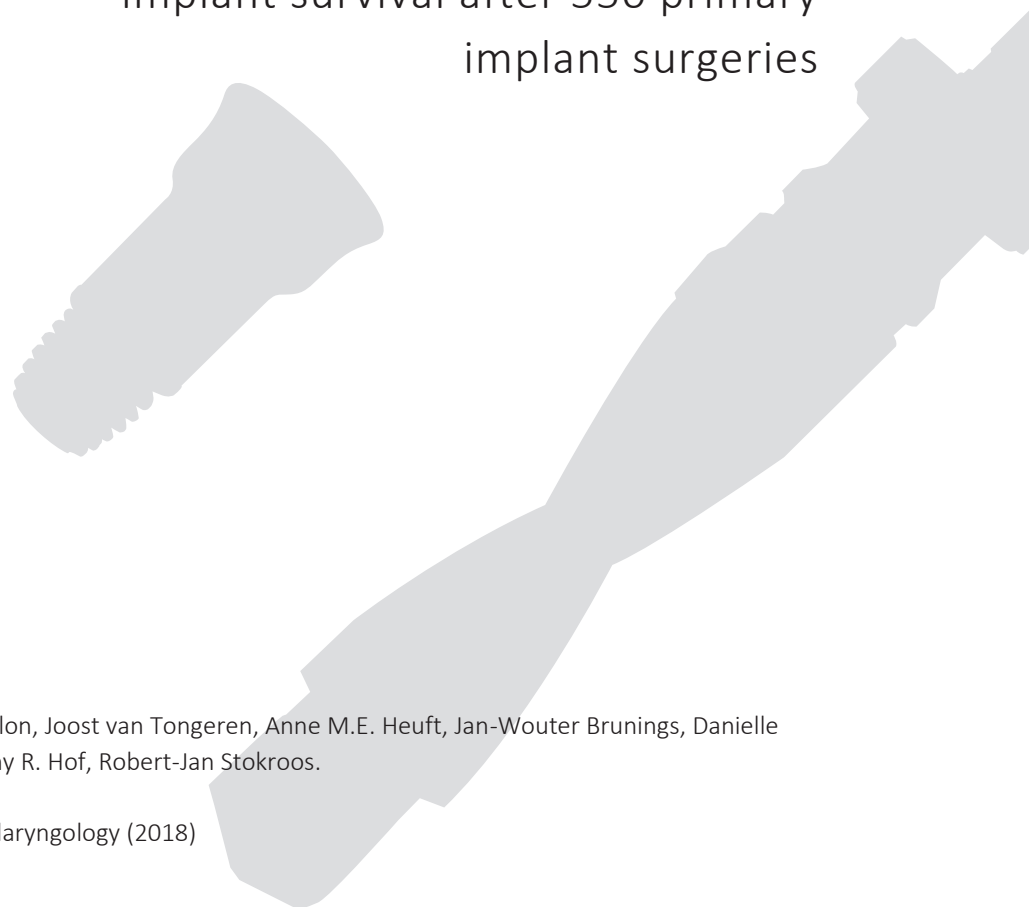
*Implant loss was reported as a SAE, based on local guidelines. According to ISO14155 guidelines, implant loss would not be defined as an SAE.



S6 ISQ values at surgery. Box plot of ISQ measurements at surgery for 9 mm and 12 mm abutments. Dots indicate the implants lost during the 12-week follow-up period.

Chapter 4

Percutaneous bone-anchored hearing system implant survival after 550 primary implant surgeries



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Clinical Otolaryngology (2018)

Introduction

The Bone Anchored Hearing System (BAHS) has become an established option for rehabilitation of several type of hearing impairment such as conductive hearing loss, mixed hearing loss and single sided deafness [1]. Overall good outcomes have been reported. Nevertheless, complications such as inflammation of the skin around the percutaneous abutment, pain and implant loss are related to BAHS [2].

For implant loss stability, primary and secondary stability are important concepts. Primary stability is defined as implant stability immediately after surgery. Dental studies show that primary stability is influenced by implant design, surgical technique, bone quantity and bone quality[3]. Secondary stability is defined as stability over time and is determined by primary stability and osseointegration. In dental implants, osseointegration is influenced by surgical trauma, implant design, smoking status and other subject related factors such as diabetes and hygiene [4].

In BAHS, implant loss rates of 8.3-18% have been reported [5–8]. 3-mm implants, young age, age of 60 or higher and male status have been described as risk factors for implant loss[5–8]. In this study, we aimed to analyze implant survival rates for BAHS surgery including risk factors for the population in Maastricht University Medical Centre+ (MUMC), The Netherlands.

Materials and Method

Ethics

Due to the retrospective nature of this study and anonymization of data ethical approval was not required according to the Medical Research Involving Human Subjects Act in the Netherlands.

Study design

This is a retrospective case study of subjects receiving a BAHS implant between 1991 and January 2017 in MUMC. A database containing all subjects that have received a BAHS implant was used. Implant length, abutment length, manufacturer and if applicable extrusion or explant surgery are captured in this database. The database was checked by a second researcher for inconsistencies.

Statistical analysis

Statistical analyses were performed using R version 3.3.2 (R Foundation for Statistical Computing, Austria). Statistical significance was established at $p \leq 0.05$. Mean (*M*) age at implantation, standard deviation age (SD), mean follow-up time and SD for follow-up

were calculated for all implants. Kaplan-Meier curves were created for overall survival. 1 -year, 5-year, 10-year and 15-year implant survival rates were calculated for primary placed 4-mm implants, 3-mm implants and for 4-mm implants placed after implant loss. Based on previous reported possible risk factors [5–8], the effect of 3-mm implants, second 4-mm implant, male sex, young age (<18) and age >60 at implantation was examined in a multivariable analysis using a Cox's proportional hazards regression model. An explorative analysis including new generation implants with a wide diameter (4,5mm) as an additional factor was examined. Hazard ratios (HR) and 95% confidence intervals (CI) were determined for all factors.

Results

Descriptives

From 1991 to January 2017, 536 subjects were implanted with 550 primary BAHS implants at MUMC. 511 4-mm (92.9%) implants and 39 3-mm (7.1%) implants were inserted in 536 subjects of which 266 (49.6%) were males and 270 females (50.4%). Mean age was 49 years (SD=18) with a mean follow-up time of 7.48 years (SD=5.0). 511 4-mm implants were inserted with a mean follow-up time of 7.5 years (SD=5.1). Mean age at implantation for 4-mm implants was 51 (SD=17). 39 3-mm implants were placed with a mean follow-up time of 5.53 years (SD=3.8). Mean age at implantation for 3-mm implants was 28 (SD=24). In 29 subjects, a total of 36 sleeper screws were placed (*M*=11.5 years of age, SD=13.7). 180 new generation wide implants were implanted as primary implant. For 7 sleeper screws (19%) were mounted with an abutment after implant loss. None were extruded during follow-up.

Implant loss

In total, 34 initial implants (6.2%) were lost during follow-up. For the primary placed implants, 28 4-mm implants (5.5%) were lost at a mean follow-up time of 3.8 years (SD=3.9). Six primary placed 3-mm implants (15.4%) were lost at a mean follow-up time of 0.99 years (SD=0.82). In 19 subjects, new implants were placed after loss of the primary implant of which 18 (95%) were 4-mm implants and 1 (5%) was 3-mm. Of the second implants 4 (21%) were lost during follow-up. Reasons for implant loss are presented in Table 1.

Most implants were lost during the first 18 months of follow-up. Spontaneous loss, trauma to the implant and inflammation were reported as reasons for implant loss. Spontaneous loss was reported for 15 male subjects compared to 10 female subjects. Trauma was observed as a reason for primary implant loss in 5 male subjects compared to 1 female subject. Elective removal was performed in two cases due to chronic pain in one case and recurrent irritation of the skin after abutment removal in the other case. After a second implantation, most implants were lost in the first 6 months.

Table 1: Causes for implant loss

	Reason	0-6 months n (%)	6 months-18 months n (%)	18 months – 5 years n (%)	> 5 years n (%)	Total n (%)
Primary placed 3-mm (n=6)						
	Spontaneous	1(16.6%)	1 (16.6%)	2 (33.3%)		4 (66.7%)
	Trauma		1(16.6%)			1(16.6%)
	Inflammation	1(16.6%)				1 (16.6%)
Primary placed 4-mm (n=28)						
	Spontaneous	6 (21%)	4 (11.5%)	3 (10.7%)	6 (21%)	19 (67.9%)
	Trauma	1 (3.6%)	1(3.6%)	2 (7.1%)	1 (3.6%)	5 (17.9%)
	Inflammation	2(7.1%)	1 (3.6%)			3 (10.7%)
	Elective removal				1 (3.6%)	1 (3.6%)
Second 4-mm after implant loss (n=4)						
	Spontaneous	2 (50%)				2 (50%)
	Trauma	1 (25%)				1 (25%)
	Elective removal			1 (25%)		1 (25%)

Survival

Kaplan Meier survival curves are presented in Figure 1. Implants survival rates are described in Table 2. Due to the limited sample size, it was not possible to calculate 10-year survival rates for the second 4-mm implant and 15-year survival rates for 3-mm implants. For the primary 4-mm implant 1-year, 5-year, 10-year and 15-year survival rates were 98%, 96%, 94% and 92% respectively. For the primary 3-mm implant, survival rates were 92%, 84% and 84%. For the second 4-mm implant placed after initial implant loss survival rates were 89% and 69%.

Cox proportional hazard models revealed that male sex (HR=1.99, 95%CI=1-3.95, p=<0.05), young age (HR=3.43, 95%CI=1.38-8.52, p=0.008) and second implant (HR=5.67, 95%CI=1.94-16.54, p<0.002) were associated with an increased risk for implant extrusion (Table 3). No significant risk at implant loss was observed for 3-mm implants (p=0.32) or subjects older than 60 years of age (p=0.41). The explorative analysis including implant diameter in the model showed similar results. No significance for increased survival for the new generation implants (HR=1.85, 95%CI=0.83-4.14, p=0.13) was observed.

Table 2: Implant survival rate

Implant Survival	Mean	95% confidence interval	
1-year			
3-mm implant (n=36)	0.92	0.84 -	1.00
4-mm implant (n=467)	0.98	0.97 -	0.99
2nd 4-mm implant (n=13)	0.89	0.76 -	1.00
5-years			
3-mm implant (n=21)	0.84	0.74 -	0.97
4-mm implant (n=310)	0.96	0.95 -	0.98
2nd 4-mm implant (n=6)	0.69	0.46 -	1.00
10-years			
3-mm implant (n=5)	0.84	0.71 -	0.97
4-mm implant (n=172)	0.94	0.91 -	0.96
15-years			
4-mm implant (n=37)	0.92	0.87 -	0.95

Table 3: Cox regression analysis of risk factors for implant loss. 3 mm implant is compared to 4 mm implant. Male gender is compared to female gender. Age > 60 is compared to age ≤ 60 years. Age < 18 years is compared to Age ≥ 18 years. Second implant after implant loss is compared to the first 4-mm implant.

Implant loss	Hazard ratio	95% confidence interval	p-value
3-mm implant	1.68	0.61-4.67	0.32
Male gender	1.99	1.00-3.95	<0.05
Age < 18 years	3.43	1.38-8.52	0.008
Age > 60 years	0.59	0.31-1.61	0.41
Second 4-mm implant	5.67	1.94-16.54	<0.002

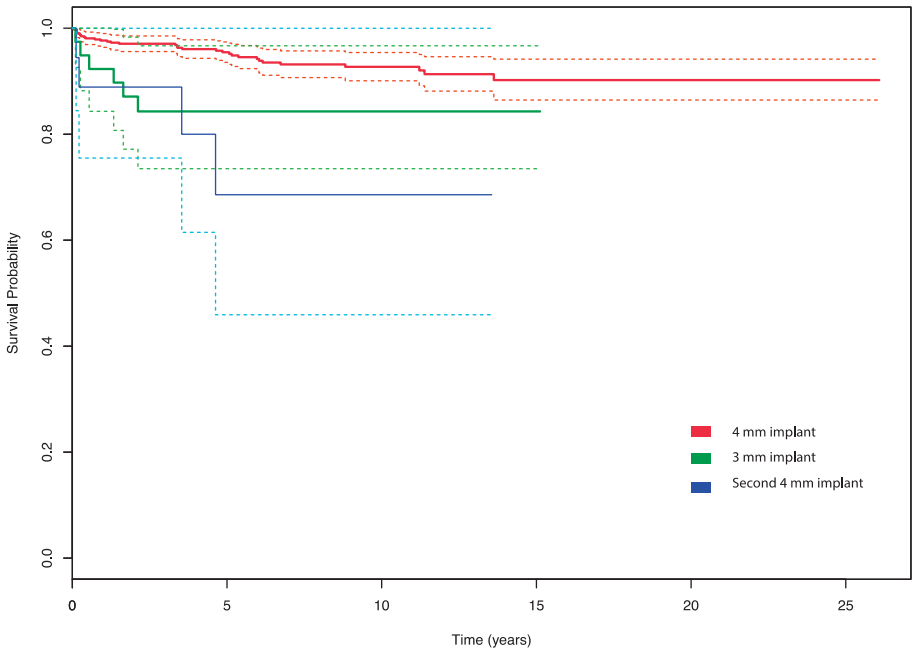

Figure 1: Kaplan Meier Implant survival curve for 1-, 5-, 10- and 15-year implant survival for primary 3-mm implants, primary 4-mm implants and second 4-mm implants after loss of the primary implant. Dashes indicate 95% confidence interval.

Discussion

Synopsis of key/new findings

This study shows an overall survival rate of 93,8% for first implants, which is high compared to previous studies[5–7]. Implant survival rates at 1-year, 5-years, 10-years and 15-years were 98%, 96%, 94%, 92% for 4-mm implants respectively. Young age, male sex and the second 4-mm implant were significantly associate with implant loss. 3-mm implants and old age were not associated with implant loss. The observed higher rates of implant loss in 3-mm implants is most likely attributed to young age. Sleeper screws are often placed in young children. After implant loss, a second sleeper screw was placed in several subjects upon abutment placement on the original sleeper screw. None of these implants was lost during follow-up possibly indicating that placement of a second sleeper screw may not be necessary in these cases.

Strengths and limitations

In this study, long term implant survival is described for 550 primary placed implants and 19 implants placed after loss making it one of the largest case series published to date for BAHS [5–7]. Moreover, here we specifically describe the use of sleeper screws. The methods used in this study provide a statistical model for risk factors related to implant loss. This study suffers from some limitations. Due to the limited number of implant losses we were unable to include factors such as very young age (<6 years), surgeon, learning curve, implant type and abutment length. Some subjects might not report an implant loss. Non-usage is not reported in our database. Possible relevant factors such as smoking status, body mass index, medication use and medical history was not structurally reported. The sample sizes of 3-mm implants, sleeper screws and second implants are relatively small warranting some caution when interpreting the data.

Comparison with other studies

Compared to other studies, the implant survival rates are high. Especially, long term follow-up results show higher survival rates. Larssen *et al.* showed 10-year survival rates for 4-mm implants was 74% and overall cohort survival rate of 91.2% [6]. Dun *et al.* described an overall cohort survival rate of 91.7%[5]. Both centers were early adopters of the BAHS system [5,6]. During the early years of development, implant loss rates may have been higher. In recent years, wider implants have been introduced [9,10] which increased stability and facilitated the use of longer abutments and possibly early loading. These wider implants may have improved 5-year survival rates. However, we did not observe this effect in our sample. Here we mainly observed trauma in male subjects. In addition to risk factors such as smoking, male subjects may potentially exhibit more active behavior, potentially explaining the increased risk for implant loss. In contrast to previous studies, we

observed no increased risk for 3-mm implants. In young subjects, 3-mm implants are often placed. An interaction between 3-mm implants and younger subjects may be present as well. The sample size of very young children is limited in our database and pooling data of several centers might be necessary to achieve an adequate sample size to identify whether very young age (<6) itself is an additional risk factor. We found no evidence for an increased susceptibility for implant loss in older subjects. Osseointegration is insufficient in cases of spontaneous loss and loss due to recurrent infection. In normal healthy bone, osseointegration should be sufficient after approximately 3 weeks to facilitate loading of the BAHS[5,10]. Often spontaneous loss is mentioned after this period indicating that some amount of osseointegration did take place. Future studies may clarify the reasons for spontaneous implant loss and their respective relation to known risk factors.

Conclusion

This study shows an overall survival rate of 93,8% for first implants. Survival rates of 98% and 92% after 1-year and 15-years follow-up respectively were found for 4-mm implants. Age <18, male gender and second implantation were found to be significantly associated with implant loss.

Conflict of interest

T.C is involved in a multicenter randomized controlled study comparing bone anchored hearing implants sponsored by Oticon Medical AB (Askim, Sweden). The other co-authors have no conflicts of interest in connection with this article.

Acknowledgements

We would like to thank Miranda Janssen (Maastricht University) for her statistical support.

Keypoints

- Overall BAHS Implant survival rate can be as high as 92% at 15-year follow-up for 4-mm implants
- Young age (< 18) is associated with increased risk for implant loss
- 3-mm implant are not associated with increased risk for implant loss
- Second implants placed after implant loss are associated with increased risk for implant loss
- Male gender is associated with increased risk for implant loss

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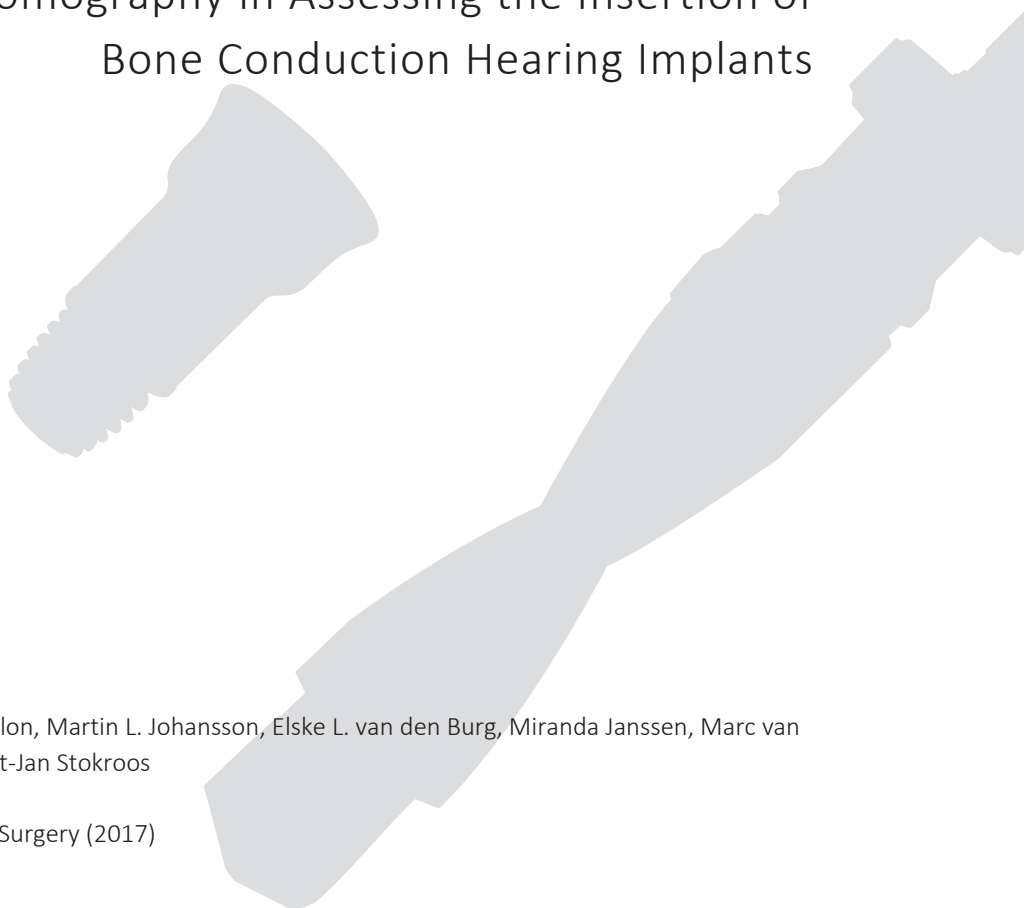
Part II

Understanding clinical outcomes

Chapter 5

The Use of Cone Beam Computed Tomography in Assessing the Insertion of Bone Conduction Hearing Implants



Tim. G.A. Calon, Martin L. Johansson, Elske L. van den Burg, Miranda Janssen, Marc van Hoof, Robert-Jan Stokroos

Frontiers in Surgery (2017)

Abstract

Objective: This study aimed to compare post-operative Cone Beam CT (CBCT) imaging to Implant Stability Quotient (ISQ) measurement and direct caliper measurements as a suitable technique to assess Bone Conduction Hearing Implant (BCHI) seating and insertion depth.

Methods: *In vitro*, BCHIs were completely (n=9) and partially inserted (n=9) in bone blocks of different densities and subsequently scanned. Scans were processed using 3DSlicer 4.3.1 and Mathematica 10.3. ISQ measurements were obtained for all BCHIs mounted with different abutment lengths (9 mm, 12 mm, 14 mm). CBCT imaging was performed for patients with a clinical indication.

Results: *In vitro*, 95% prediction intervals for partially inserted and completely inserted BCHIs were determined. ISQ values significantly decreased with partial insertion, low-density artificial bone and longer abutment lengths. Evaluation of *in vitro* and *in vivo* 3D models allowed for assessment of insertion depth and inclination.

Conclusion: CBCT imaging allows to study implant seating and insertion depth after BCHI surgery. This can be useful when visual confirmation is limited. It is possible to distinguish a partial BCHI insertion from a complete insertion in artificial bone blocks. This technique could prove to be a valuable research tool. *In vitro*, ISQ values for Ponto BCHIs relate to abutment length, insertion depth and artificial bone density.

Keywords: Bone anchored hearing implants, radiology, BAHA, Osstell, Implant stability, Resonance frequency analysis

Introduction

In recent years, surgical placement of bone conduction hearing implants (BCHI)s has become less invasive with the introduction of the linear incision technique with tissue preservation (1). In an attempt to further improve outcomes, punch only techniques have been described with good initial results (2–4). In line with these developments the punch only Minimally Invasive Ponto Surgery (MIPS) technique was recently introduced (5,6) to standardize this procedure.

During BCHI surgery, the implant should be placed perpendicular to the skull to allow for full and straight insertion. Using conventional techniques, the bone bed is visible which allows for visual feedback regarding insertion depth and implant angle. However, with tissue preservation, surgical technique visibility is reduced and with punch only techniques visual confirmation is even further obstructed by the surrounding tissue. In absence of visual confirmation, we sought to obtain an objective feedback tool to verify complete insertion and angulation.

Imaging techniques might provide objective feedback regarding insertion depth and angulation of BCHI seating. However, traditional plain radiography techniques cannot show the necessary 3D detail and conventional CT imaging is difficult to justify due to its radiation burden and scattering sensitivity. Cone Beam CT (CBCT) is characterized by high resolution, reduced sensitivity to scattering artifacts, lower overall radiation doses compared to conventional CT imaging. CBCT imaging has been described as an appropriate technique for the evaluation of peri-implant bone for dental implants and cochlear implant position (7–10).

Another possibility to assess implant insertion is the Implants Stability Quotient (ISQ), a non-invasive method based on Resonance Frequency Analysis (RFA) (11). This method is being propagated as a method to assess implant stability in temporal bones (12). ISQ measurements can be obtained during surgery, possibly allowing direct intra-operative feedback and intervention. Although ISQ values are regularly reported in BCHI studies (12–14), a high level of uncertainty surrounds the clinical utility of ISQ for BCHIs. Limited consistent information is available on the multivariable interplay of clinically relevant or irrelevant factors for BCHIs. Factors described in dental studies include: drilling protocol, abutment length, abutment morphology, abutment weight, implant design and surface morphology, bone density, bone to implant contact, and most importantly: osseointegration (15). Moreover, how and to what extent, ISQ measurements are affected by incomplete or angulated insertion is unknown. Before ISQ measurements can be used as a clinical diagnostic, studies proving unambiguous interpretation and validity are needed. At the moment, these are not available.

This explorative study aimed to investigate if post-operative CBCT imaging is a suitable technique to assess BCHI seating and insertion depth either *in vitro* or *in vivo*. *In vitro* validation of this technique was done in an experimental setting. In the clinical part of this study, CBCT imaging of the BCHI was performed in patients when the surgeon was uncertain

about either insertion depth or insertion angle. To interpret *in vivo* seating, we matched these retrospectively to our *in vitro* results. Additionally, it was determined how ISQ values are affected by degree of insertion, abutment length and artificial bone density *in vitro*.

Materials and methods

Ethics

The procedures in this study were in accordance with legislation (the Medical Research Involving Human Subjects Act) and ethical standards on human experimentation in the Netherlands. CBCT scans were made to assess BCHI seating on clinical indication. According to the Medical Research Involving Human Subjects Act (WMO), ethical approval was not required due to the nature and anonymization of the data.

Imaging analysis

All CBCT scans were acquired using the I-CAT scanner (Imaging Sciences International, Hatfield, Pennsylvania, United States of America) with 0.125 mm isometric resolution. Tube current was 37.07 mAs with a tube voltage of 120Kv. A full rotation took 26.7 seconds. Scans were processed with 3D Slicer 4.3.1 (http://slicer.org). A region of interest (ROI) was selected, containing the BCHI and (artificial) bone adjacent to the BCHI. Fixed threshold grey-level values were used to create segmented volumes of the BCHI, (artificial or real) bone and soft tissue. The segmented volumes were imported in Mathematica 10.3 (Wolfram Research, Champaign, United States of America) to create 3D models.

In vitro validation

Artificial bone blocks

BCHIs were installed in polyurethane artificial bone blocks (13 cm x 8.8 cm x 4 cm) with different densities (Sawbones, USA) (Figure 1A). Two high density (50 pounds per cubic foot) and one low density (40 pounds per cubic foot) artificial bone blocks were created. Installation of 4 mm Wide Implants (Oticon Medical AB, Askim, Sweden) mounted with 14 mm abutments (Oticon Medical AB, Askim, Sweden) were carried out using the surgical instrument designed for MIPS (5). Implants were fully inserted with 4.5 rotations or partially inserted with 3.5 rotations (Figure1B).

BCHI insertion measurements

Virtual insertion depth measurements on the CBCT and direct manual caliper measurements were completed for 18 implants placed in three bone blocks (Figure 1C). According to specifications provided by the manufacturer, the distance from the top of the abutment (14 mm) and the implant rim should range between 14.43 mm and 14.59 mm when fully inserted. The middle (14.51 mm) was used as a comparative reference value for full insertion in the analysis. In 3D Slicer virtual markings were placed indicating the highest point of the abutment top, the bottom of the implant rim and bone surface in four quadrants (Figure 1D). Per quadrant, the virtual bone surface to abutment top distance and the virtual bone surface to implant rim distance were calculated. Manual caliper measurements were obtained at every quadrant from the abutment top to the level of the artificial bone and thereafter averaged per implant. The distance of the implant rim to artificial bone was estimated by subtracting the abutment length from the caliper measurements.

ISQ measurements

The Osstell ISQ (Ostell, Gothenburg, Sweden) was used to measure ISQ values by mounting a Smartpeg Type 55 on the abutments. Two perpendicular ISQ measurements (ISQ Horizontal, ISQ vertical) were obtained for all implants (12). To test the influence of abutment length, ISQ values were obtained for each implant mounted consecutively with 9 mm, 12 mm and 14 mm abutments, resulting in 6 measurements per implant. Twelve implants were tested for the high density bone configuration, half of them fully seated and half partially seated. For the low density bone configuration six implants were placed, half fully seated and half partially seated (Figure 1C). For the high density bone configurations, one fully and one partially seated implant moved during abutment replacement. Consequently, in high density bone 10 BCHIs were evaluated (n=5 fully seated, n=5 partially seated) (Figure 1C). Due to gross implant mobility during abutment replacement, it was not possible to change the abutment in the low-density artificial bone block that was implanted with partially inserted BCHIs, hence only fully inserted BCHIs were tested (n=3).

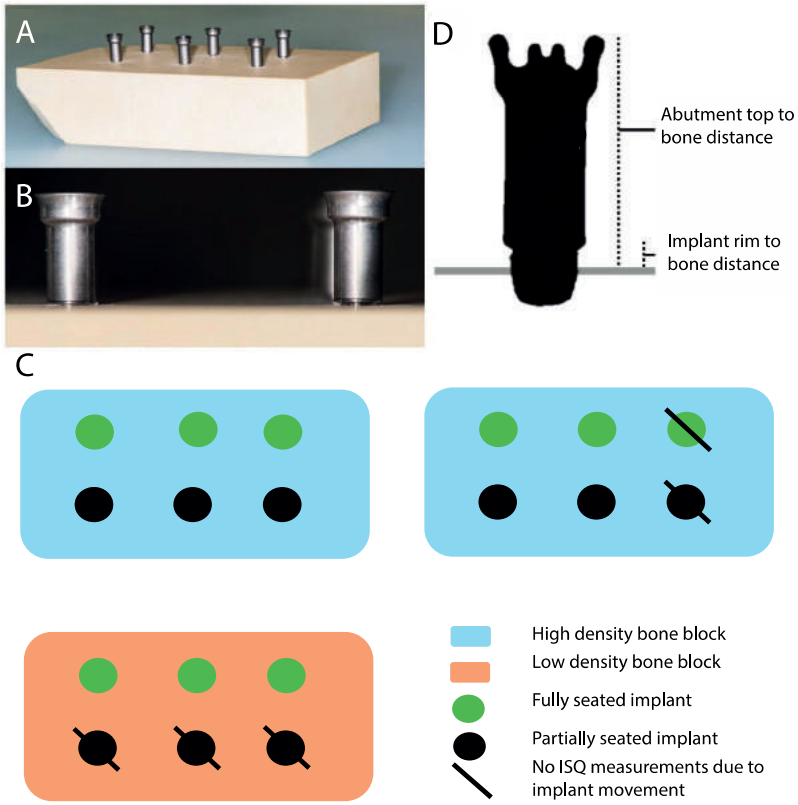


Figure 1: Overview of the system and the measurement points. (A) Overview of artificial bone block with installed implant. (B) Overview of fully seated BCHI (left) and partially seated BCHI (right). (C) Overview of artificial bone blocks. BCHI insertion measurements were performed on all bone blocks. \ indicates implants not used for ISQ measurements due to gross implant mobility. (D) Coronal cross section plane of the BCHI. Measurement points are placed at the abutment top, bottom of the implant rim and (artificial) bone at four equally spaced quadrants. In this exemplary case, the implant is partially seated.

In vivo application

After *in vitro* validation, CBCT scans were retrieved retrospectively from subjects in the outpatient clinic of Maastricht University Medical Center. The scanning protocol was identical to the *in vitro* scans. Subjects were included when a CBCT scan was performed in a clinical setting to evaluate post-surgical implant seating.

Statistical analysis

Statistics were performed using SPSS software (SPSS V22.0 SPSS Inc., Chicago, Illinois). Statistical significance was established at $p \leq 0.05$.

In vitro-BCHI insertion measurements

Analyses were used for *in vitro* validation of CBCT imaging before they could be applied *in vivo.* For direct caliper measurements and CBCT measurements mean (*M)* and standard deviation (SD) for full insertion and partial insertion were calculated for abutment top to bone surface distances and implant rim to bone surface distances. Mean distances for abutment top to bone surface of CBCT measurements and caliper measurements for full and partial insertion were compared to each other and to the theoretical average for full insertion. Regression analysis in which the CBCT abutment top to bone surface distance and CBCT implant rim to bone surface distance were regressed to insertion depth (full or partial) was performed. 95% Prediction interval for fully inserted and partially inserted BCHIs were determined. Discriminant analysis with equal prior probabilities was applied to determine the optimal cut-off point for classification of BCHIs as fully or partially inserted.

In vitro - ISQ measurements

For ISQ measurements, linear mixed model analyses with implant as random factor and abutment length (9 mm, 12 mm, 14 mm), artificial bone density (high, low), and insertion depth (full, partial) as fixed factors and a compound symmetry covariance matrix of the residuals. Because in the low-density artificial bone block only fully inserted BCHIs could be measured, the effect of insertion depth could only be determined at high bone density. Similarly, the effect of bone density could only be determined for fully inserted BCHIs. When statistically significant effects were identified, Bonferroni adjusted post hoc comparisons between the different factor levels were additionally performed.

Results

In vitro

BCHI insertion measurements

Direct caliper measurements from abutment top to the bone surface of fully inserted BCHIs (*M*=14.62, SD=0.06, n=9) were 0.11 mm greater than the theoretical average for full insertion *in vitro* (Figure 2A). CBCT measurements of fully inserted BCHIs (*M*=14.83, SD=0.08, n=9) were on average 0.32 mm greater than the theoretical average for full insertion *in vitro.* For the partially inserted implants, direct caliper measurements (*M*=15.06, SD=0.09, n=9) and CBCT measurements (*M*=15.27, SD=0.10, n=9) were both 0.44 mm greater compared to the fully inserted BCHIs.

The implant rim to artificial bone surface distances as measured on CBCT were 0.47 mm larger in partially inserted BCHIs (*M*=0.73, SD=0.07, n=9) compared to fully inserted BCHIs (*M*=0.26, SD=0.09, n=9) (Figure 2B).

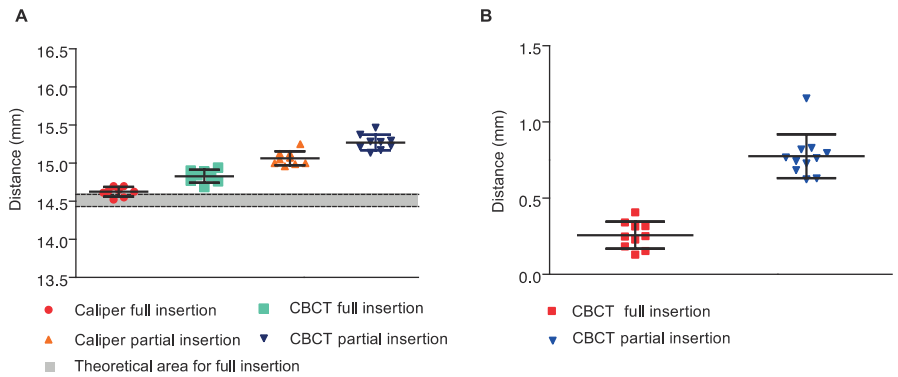


Figure 2: Measurements between respectively the abutment top or implant rim and artificial bone *in vitro.*

Mean (±SD) are displayed for fully (n=9) and partially (n=9) inserted 14mm BCHIs. (A) Distances between implant top and artificial bone surface as measured using a caliper or performed digitally on CBCT scans. The approximated theoretical area for full insertion according to the manufacturer specifications is displayed in grey. (B) Distances between the implant rim and artificial bone surface as virtually measured on CBCT.

Regression analyses revealed that the type of artificial bone was a significant predictor for abutment top to bone surface distance ($p<0.015$). 95% Prediction intervals for high density, low density and the combined group are presented in Table 1. For implant rim to bone surface no significant difference between high and low density was found. By means of discriminant analysis, cut-off points were obtained which could potentially be used to classify BCHI's either as fully or partially inserted (Table 1). Mean abutment top to bone surface distance for fully inserted BCHIs in high density bone was 14.85 mm (SD=0.06, n = 6) compared to 14.78 mm (SD=0.10, n=3) for fully inserted BCHIs in low density bone resulting in a difference of 0.07 mm. Mean abutment top to bone surface distance for partially inserted BCHIs in high density bone was 15.32 mm (SD=0.09, n=6) compared to 15.18 mm (SD=0.05, n=3) for partially inserted BCHIs in low density bone resulting in a difference of 0.14 mm.

Table 1: Prediction intervals and cut-off points.

	CBCT abutment top to bone surface distance			CBCT rim to bone surface distance		
	95% prediction interval		cut-off point	95% prediction interval		cut-off point
High, full insertion	14.67	15.04	15.09	0.04	0.39	0.47
High, partial insertion	15.14	15.50		0.56	0.91	
Low, full insertion	14.53	15.04	14.98	0.13	0.57	0.53
Low, partial insertion	14.92	15.44		0.50	0.94	
Combined, full insertion	14.63	15.03	15.05	0.07	0.44	0.49
Combined, partial insertion	15.07	15.48		0.54	0.91	

Results for regression analysis. High: high density bone. Low: low density bone Full: 4.5 rotations. Partial: 3.5 rotations.

In vitro 3D models

Evaluation of the in vitro 3D models allowed for a detailed qualitative assessment of the implant, artificial bone and the implant-artificial bone interface. BCHI insertion and angulation could be evaluated as well in the axial, coronal or sagittal plane allowing to visually distinguish between partially and fully inserted BCHIs (Figure 3). Softening effects were visible directly under the BCHI in the artificial bone models (Figure 3C).

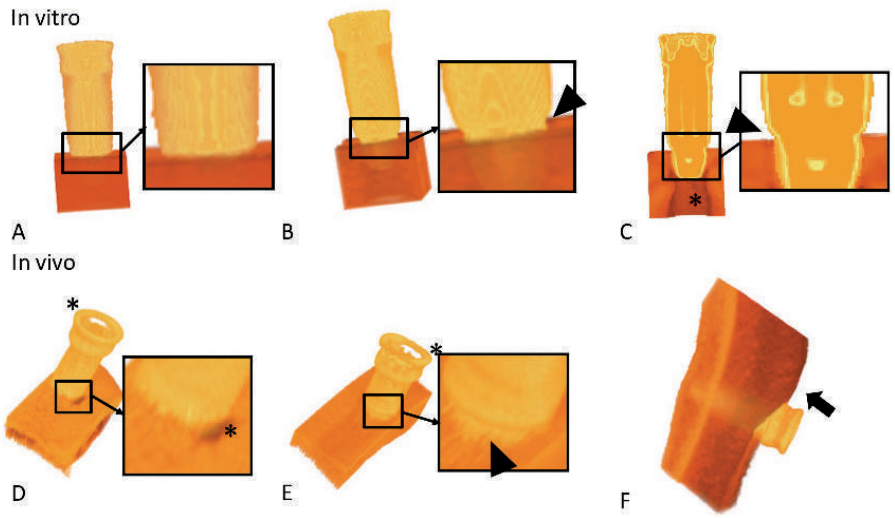


Figure 3: 3D segmented models of BCHIs acquired by CBCT imaging. *In vitro* models: (A) 3D model of a fully inserted BCHI. Full insertion is qualitatively verified by the lack of gap between the implant rim and the artificial bone surface. (B) In contrast, Figure B shows a 3D model of a bone surface is visible (arrowhead). (C) A cross sectional model of a partially inserted BCHI where a gap between the implant rim and artificial bone surface is visible (arrowhead). *In vivo* models: (D) A 3D model of the implant-skull interface in Subject 1 (full insertion). The skin was removed using a filter. (E) A 3D model of the implant skull-interface in Subject 4 showing an angulated (full insertion on one side, distance between implant rim and surface on the opposing circumference (arrowhead)). The skin was removed using a filter. (F) A 3D model of the bone, implant and soft tissue interface for Subject 1. Skin sagging was present and can be discerned (arrow). Asterisks (∗) indicate softening artefacts.

ISQ measurements

ISQ measurements are shown in Figure 4 and the models are presented in Table 2. In a split plot ANOVA analysis, the ISQ in high density artificial bone revealed a significant negative relationship with abutment length ($p<0.001$) and insertion depth ($p=0.001$), whereas no interaction between abutment length and insertion depth was found ($p=0.378$) (Table 2). To evaluate the effect of bone density a linear mixed model analysis was performed using the ISQ data of the fully inserted BCHIs. After removing the non-significant interaction ($p=0.153$) between abutment length and bone density, abutment length ($p<0.001$) and bone density ($p=0.024$) were both significant predictors of ISQ (Table 2).

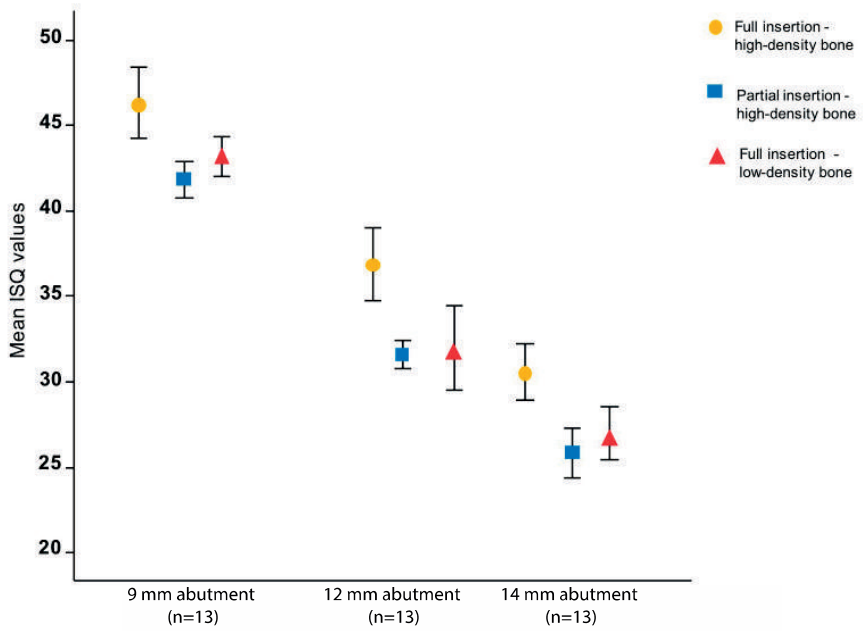


Figure 4: The influence of abutment length, bone density and insertion on ISQ in vitro. The mean ISQ values for high-density bone configurations (N=5) and low-density bone configuration (N=3) together with the standard deviations are plotted for different abutment lengths and artificial bone densities. ISQ values decrease significantly with increase in abutment length ($p<0.001$), partial insertion ($p=0.001$) and reduced bone density ($p=0.024$). ISQ measurements of low density partially inserted implants are missing due to an interaction between low density and partial insertion (Figure 1C).

Table 2: Two mixed models for ISQ.

ISQ for high density artificial bone (n=10 implants)					
	Estimates	95% confidence interval		Degrees of Freedom	p
Intercept*	46.47	44.96	47.97	9.10	<0.001
Insertion					
Full (n=5)	-	-	-	-	-
Partial (n=5)	-4.83	-6.93	-2.73	8.00	<0.001
Abutment length					
9 mm (n=10)	-	-	-	-	
12 mm (n=10)	-9.8	-10.41	-9.19	18.00	<0.001
14 mm (n=10)	-15.8	-16.41	-15.19	18.00	<0.001
ISQ for high and low density artificial bone (n=8 implants)					
	Estimates	95% confidence interval		Degrees of Freedom	p
Intercept**	46.58	44.62	48.54	7.36	<0.001
Abutment length					
9 mm (n=8)	-	-	-	-	-
12 mm (n=8)	-10.06	-11.04	-9.08	14.00	<0.001
14 mm (n=8)	-15.88	-16.85	-14.90	14.00	<0.001
Bone density					
High (n=5)	-	-	-	-	-
Low (n=3)	-3.88	-7.05	-0.70	6.00	0.024

Results for 2 Mixed models. ISQ for high density artificial bone model contains insertion depth and abutment length. Density was not used in this model because only fully inserted BCHIs could be measured in the low density bone blocks. ISQ for High and Low density artificial bone model contains bone density (High/Low) and abutment length. *Intercept indicates reference value for full insertion implant mounted with 9 mm abutment. ** Intercept indicates reference value for implant mounted with 9 mm abutment in high density bone. – indicates reference variable.

In vivo

Subjects

Four subjects were selected one-week post-surgery at the ENT out-patient department of Maastricht University Medical Center to evaluate BCHI with a clinical indication. Subject characteristics are summarized in Table 3.

Table 3: Subject characteristics and CBCT imaging indications.

Subject	Demographics	Indication	Implant type	Surgical technique	Reason for CBCT
1	71-year old male	Mixed hearing loss	Ponto Wide implant with 12 mm abutment	Linear incision with soft tissue preservation technique	Evaluation of BCHI seating one week post-surgery
2	32-year-old male	Conductive hearing loss	Ponto Wide implant with 9 mm abutment	Linear incision with soft tissue preservation technique	Evaluation of BCHI seating one week post-surgery
3	63-year-old male	Mixed hearing loss	Ponto Wide implant with 14 mm abutment	MIPS technique	Evaluation of BCHI seating one week post-surgery
4	51-year-old female	Conductive hearing loss	Ponto Wide implant with 9 mm abutment	MIPS technique	Evaluation of BCHI seating one week post-surgery

BCHI insertion measurements

CBCT measurements are described in Table 4. Distances between the implant rim and bone surface were consistent with a full insertion (0.49 mm) for subjects 1, 2 and 3. For subject 4, the measurements per quadrant were 1.0 mm, 0.09 mm, 0.07 mm and 0.37 mm respectively, resulting in a mean distance of 0.38 mm. The mean distance was consistent with full insertion, but the distance of 1.0 mm for one quadrant was greater than the cut-off value of 0.49 which could indicate an angulated insertion (See 3D models).

We assumed that the abutment top to bone surface cut-off distances for the 14 mm abutments could be adjusted for 9 mm and 12 mm abutments. The cut-off value for 14 mm abutments (15.05 mm) was therefore adapted for 9 mm abutments (10.05 mm) and 12 mm abutments (13.05 mm). Distances between abutment top and bone were consistent with full insertion in all subjects.

Table 4: CBCT measurements.

Subject	Abutment length	Mean abutment top to bone distance (cut-off value)	Mean implant rim to bone distance (cut-off value)	Interpretation
1	12 mm	12.45 mm (13.05 mm)	0.06 mm (0.49 mm)	Full, straight insertion
2	9 mm	9.48 mm (10.05 mm)	0.05 mm (0.49 mm)	Full, straight insertion
3	14 mm	14.11 mm (15.05 mm)	0.16 mm (0.49 mm)	Full, straight insertion
4	9 mm	9.63 mm (10.05 mm)	0.38 mm (0.49 mm)	Full, angulated insertion*

* Visually assessed

3D models

Evaluation of the *in vivo* 3D models allowed for qualitative assessment of the BCHI, bone and soft tissue, allowing visual appraisal of implant seating and angulation (Figure 3). In the in vitro 3D models, the complete abutment could be evaluated, while softening

effects around the abutment top made it difficult to evaluate the location of the abutment top in the *in vivo* 3D models. Qualitative evaluation of the post-surgery scans for subjects 1, 2 and 3 revealed a full non-angulated BCHI insertion (Figure 3D). In the post-surgery scan of subject 4, the 3D model revealed an angulated insertion of the BCHI (Figure 3E), which is consistent with the differences observed per quadrant. Unexpectedly, the 3D patient model also facilitated evaluation of the skin next to the implant. In subject 1 the presence of skin sagging, which entails excess skin superior to the abutment, was noticeable (Figure 3D).

Discussion

Using CBCT and image analysis software, it is possible to distinguish between a normal and incomplete insertion using CBCT measurements in vitro. Detailed 3D models were created to evaluate BCHI seating in vitro and *in vivo*. In this study, we show that ISQ values are dependent on abutment length, insertion depth and artificial bone density for Ponto implant/abutment combinations. This is consistent with other (dental) studies showing the abutment design and bone density influence (primary) stability as measured with ISQ (15).

One potential drawback with punch only surgical approaches is the lack of reference for a straight and full insertion. Surgeons may estimate implant stability using a torque wrench, but the reliability of this method is unclear. During the first punch only surgeries we used small instruments to assess complete insertion. In our experience this method is unreliable and may lead to undesirable tissue damage. The installation indicator developed for MIPS may be useful to detect incomplete insertion (5), but was not investigated here. It guides counting the number of rotations during BCHI installation indicating full insertion. The surgeon can manually complete the installation of the BCHI using a torque wrench in case of incomplete insertion. However, since the skull is not flat angulated seating may be missed using this approach.

BCHI insertion measurements

Here we demonstrated that CBCT imaging allows for the evaluation of implant insertion depth, seating and angulation in an *in vitro* setting. We assume that these results could be applicable *in vivo* as well. In the *in vitro* model, the facet of the artificial bone block was flat. In the *in vivo* situation, this is however not the case due to the curvature of the scull. Nevertheless, CBCT imaging takes the curvature of the skull into account as well as demonstrated in Figure 3D/3E. Although, we found statistically significant differences between bone blocks, these are relatively small. During insertion of an implant, *in vitro* or *in vivo*, the friction between the implant surface and surrounding bone will be influenced by the density, insertion torque and other properties of the bone. Hence, installing an implant with a specific insertion torque will likely result in a deeper inserted BCHI in soft

compared to hard bone. Currently new surgical techniques (5,6), reduced time until loading of the BCHI (16) and new implants (17) are investigated. In these and other future studies CBCT scanning techniques with image analysis might provide an opportunity for evaluation of the implant seating, implant-bone interface and possibly, temporal progression of osseointegration (8) or its decline. In this study, the diagnostic, predictive and clinical values of CBCT imaging were not determined warranting further validation studies. Pending future validation, one might imagine its use in specific clinical indications (*e.g.* post-traumatic, loose implants).

ISQ measurements

ISQ measurements are sensitive to a partial insertion might be explained by the different pivoting poit of the implant in relation to the bone level. Hypothetically this could lead to a different pendular movement, amplitude and resonance frequency induced by the (constant) force exerted by the ISQ probe upon the Smartpeg. We could not change the abutments in partially inserted implants in low density bone. How ISQ measurements should be interpreted as a clinical tool remains unclear with no validated predictive cut-off value to indicate good stability or survival. In previous studies, no correction for abutment length has been performed and usually the sample size is limited (12). Further research is necessary to investigate the multivariate role of ISQ measurements in relation to clinical outcomes.

Limitations

This study suffers from several limitations. The mixed model results provide an estimation of the effect of abutment length, done density and partial seating. Theoretically several interactions may play a role as well, which were not statistically significant in this model. These could relate to the small sample size of this investigation. Nonetheless, our results should be considered as approximations. CBCT measurements are known to under- or overestimate a distance (18,19). Overestimation of CBCT measurements compared to caliper measurements was the case here as well, although minor. The measured distance can be expected to rely heavily on the type of windowing. In our experience, each CBCT scanner has different parameters for different intensities unlike the standardized Hounsfield Units. Therefore, some caution is warranted for the implementation and translation of our results. A solution to this problem would be the development of uniformly well-calibrated CBCT scanning parameters and reconstruction for this specific setup. Another limitation is the presence of hardening and softening artefacts in the area adjacent to the implant, resulting in an impaired possibility to evaluate surface directly adjacent to the BCHI. Softening effects were objectified as well directly under the implant *in vitro* (Figure 3C) and around the abutment top *in vivo*, resulting in a distorted 3D reconstruction (Figure 3D & 3E). Due to the softening effects of the abutment top, the reliability of the abutment top to bone surface distances should be considered lower than

the implant rim to bone surface distances for these cases. Although this is unfortunate, the implant rim to bone surface distances and 3D reconstruction of the implant adjacent to the skull hold the most relevant information for determining implant seating, angulation and implant bone interface. The positioning of a subject in the CBCT scanner may influence softening effects of the abutment top and should be considered when performing a CBCT scan. Additionally, bone thickness adjacent to an implant might be underestimated due to softening artefacts. Further reduction of these hardening and softening artefacts could allow for better evaluation of the tissues adjacent to the BCHI enabling researchers to investigate implant stability *in vivo*.

Conclusion

CBCT imaging allows to study implant seating and insertion depth after BCHI surgery. This can be useful when visual confirmation is limited. It is possible to distinguish a partial BCHI insertion from a complete insertion in artificial bone blocks. This technique could prove to be a valuable research tool. *In vitro*, ISQ values for Ponto BCHIs relate to abutment length, insertion depth and bone density.

AUTHOR CONTRIBUTIONS

TC is involved in the execution and analysis of the study. MJo is involved in the design and execution of the study. MvH is involved in the design and analysis of the study. TC, MvH and MJo wrote the manuscript. EB contributed to the analysis of the results. RS supervised the study. MJ is involved in the analysis of the study. All authors reviewed and edited the manuscript.

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Conflict of interest statement

MJ is an employee of Oticon Medical AB. (Askim, Sweden)

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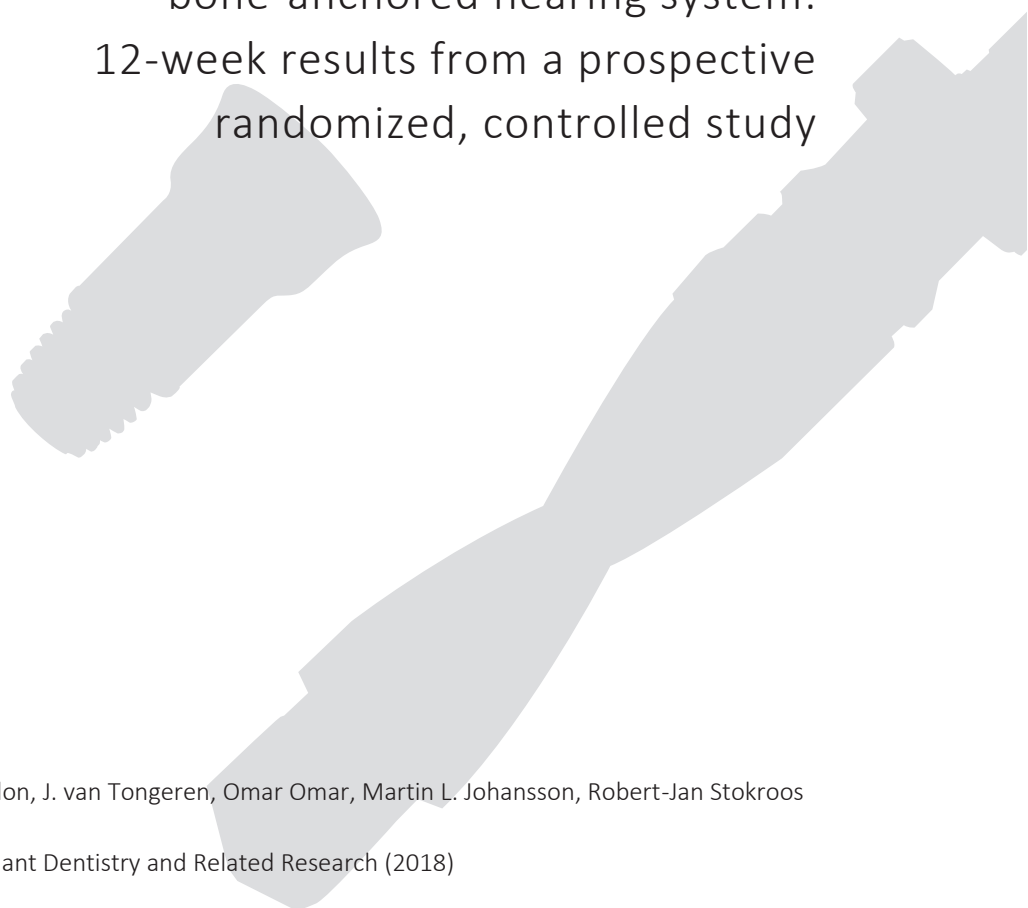
Chapter 6

Cytokine expression profile in the bone-anchored hearing system: 12-week results from a prospective randomized, controlled study



Tim G.A. Calon, J. van Tongeren, Omar Omar, Martin L. Johansson, Robert-Jan Stokroos

Clinical Implant Dentistry and Related Research (2018)

Abstract

Objective: To study the effect of implanting the percutaneous bone-anchored hearing system (BAHS) itself and inflammation of the peri-abutment skin warrant clarification. In this study, we aimed to acquire further insight into the immune responses related to BAHS surgery and peri-implant skin inflammation.

Materials and Methods: During surgery and 12 weeks post-implantation, skin biopsies were obtained. If applicable, additional biopsies were taken during cases of inflammation. The mRNA expression of IL-1β, IL-6, IL-8, TNFα, IL-17, IL-10, TGF-ß, MIP-1α, MMP-9, TIMP-1, COL1α1, VEGF-A, FGF-2 TLR-2 and TLR-4 was quantified using qRT-PCR.

Results: Thirty-five patients agreed to the surgery and 12-week biopsy. Twenty-two patients had mRNA of sufficient quality for analysis. Ten were fitted with a BAHS using the minimally invasive Ponto surgery technique. Twelve were fitted with a BAHS using the linear incision technique with soft-tissue preservation. Five biopsies were obtained during episodes of inflammation. The post-implantation mRNA expression of IL-1β ($p=0.002$), IL-8 ($p=0.003$), MMP9 ($p=0.005$), TIMP-1 ($p=0.002$) and COL1α1 ($p<0.001$) was significantly up-regulated. IL-6 ($p=0.009$) and FGF-2 ($p=0.004$) mRNA expression was significantly down-regulated after implantation. Within patients, no difference between post-implantation mRNA expression (at 12 weeks) and when inflammation was observed. Between patients, the expression of IL-1β ($p=0.015$) and IL-17 ($p=0.02$) was higher during cases of inflammation compared with patients who had no inflammation at 12-week follow-up.

Conclusions: The results of the present randomised, prospective clinical trial show that, after 12 weeks of BAHS implantation, the gene expression of some inflammatory cytokines (IL-8 and IL-1β) is still relatively high compared with the baseline, steady-state, expression. The up-regulation of anabolic (COL1α1) and tissue-remodelling (MMP-9 and TIMP1) genes indicates an ongoing remodelling process after 12 weeks of implantation. The results suggest that IL-1β, IL-17 and TNF-α may be interesting markers associated with inflammation.

Keywords: BAHA, Bone Anchored Hearing System, BAHS, cytokines, inflammation, Holgers Index

Introduction

The percutaneous bone-anchored hearing system (BAHS) has become an established treatment option for patients suffering from various types of hearing impairment. The BAHS consists of a titanium fixture, together with a pre-mounted skin-penetrating abutment, to which a sound processor can be attached.[1] The fixture is implanted in the retro-auricular temporal bone and relies on osseointegration for anchorage.[2]

A percutaneous prosthesis penetrates the skin, thereby coming into direct contact with the outer environment, and it may elicit a variety of periprosthetic tissue responses, such as inflammation and infection.[3] In fact, adverse skin reactions, such as skin overgrowth, soft-tissue reactions and infection, represent common complications.[4] Apart from discomfort and morbidity, recurrent episodes of inflammation can limit the use of the sound processor and in some cases lead to the extrusion of the fixture or voluntary implant removal. To assess the soft tissue surrounding the abutment, Holgers Index is commonly used in clinical practice and it is frequently described as an endpoint analysis in trials, although questions regarding its validity warrant further study.[5–7]

Materials implanted in hard or soft tissue stimulate different cell types. For instance, inflammatory cells produce cytokines, such as interleukin-1 (IL-1), IL-6, IL-10 and tumour necrosis factor-α (TNF-α), which are involved in regulating the immune response and wound healing. It has been hypothesised that several factors affect the biological response to implants; they include the surgical trauma, the shape and chemical characteristics of the material and the host tissue itself.[8,9] In addition, shear stress concentration in the mobile skin interfacing the rigid abutment may lead to micro-trauma and cell damage, resulting in a prolonged inflammatory state in the peri-abutment skin.[10,11] Bacterial infection has a major impact on inflammation, where the combined presence of an implant and bacteria affects the local immune response, potentially facilitating the establishment of an infection. Bacterial colonisation can be expressed as biofilm and possibly intra-cellular infection[12] that may contribute to inflammation.[13] Surgical techniques, bacterial colonisation, a foreign-body response and specific immune responses are all likely to contribute to the development of adverse soft-tissue reactions around a percutaneous abutment. The role of each of the contributory factors remains to be clarified.

The inflammatory process around skin-penetrating titanium implants is characterised the by presence of different types of leukocyte.[14] Histological and immunohistochemical analyses have revealed large numbers of inflammatory and immunocompetent cells in the area close to the abutment, suggesting that inflammatory reactions are present in the area facing the abutment, even if the skin is not clinically classified as infected [14]. Biopsies taken from patients with clinical signs of inflammation demonstrated a larger number of polymorphonuclear leukocytes, B-lymphocytes, T-lymphocytes and macrophages compared with those without clinical signs of inflammation.[14]

Several studies that correlated crevicular fluid cytokines to inflammatory conditions around dental implants, such as peri-implantitis, a destructive inflammatory process

affecting the bone and soft tissues around osseointegrated dental implants, have been published.[15] In the last review, IL-1ß and TNF-α were identified as major cytokines in relation to peri-implantitis. In contrast to dental implants, our knowledge of the cytokine expression profile in the soft tissue and peri-abutment fluid surrounding extra-oral percutaneous implants is very limited. Recently, Lennerås and co-workers described the molecular profile associated with percutaneous femoral prostheses and correlated it to different microbiological and clinical parameters, including a modified Holgers Index of the skin surrounding the abutment. Among several detected correlations, the expression of TNF-α correlated to the presence of *S. aureus* species, whereas the expression of MMP-8 correlated to polymicrobial detection. Nevertheless, no correlation was observed between the modified Holgers Index and any of the analysed genes.[16] In the field of BAHS, one study by Grant and co-workers correlated the production of peri-abutment fluid exudate and its cytokine content to Holgers Index, as an indicator of inflammation.[17] The Holgers Index scores correlated to the fluid volume, as well as with the total amounts of IL-1ß and IL-8. However, in the latter retrospective studies, the times of retrieval and analysis were relatively random in relation to implantation surgery.

The aims of this prospective clinical study were: (i) to evaluate the molecular profile of factors related to soft-tissue healing and inflammation at baseline and after 12 weeks of implantation in BAHS patients treated with either a linear incision or minimally invasive Ponto surgery (MIPS) approaches; (ii) to correlate the gene expression to clinical manifestations of soft-tissue complications, as judged by Holgers index, as well as to local and systemic factors that may affect the early outcomes of BAHS. The gene expression levels of IL-1β, IL-6, IL-8, TNF-α, IL-17, IL-10, transforming growth factor-beta (TGF-ß), MIP-1α, tissue metabolism (MMP-9, TIMP-1, collagen type 1 (COL1α1), vascular endothelial growth factor (VEGF)-A, basic fibroblast growth factor (FGF-2), Toll-like receptor (TLR)-2 and TLR-4 were determined in peri-abutment soft-tissue biopsies by quantitative real-time PCR (qRT-PCR).

Materials and Methods

Ethics

This study was performed in accordance with the Dutch legislation on Medical Research Involving Human Subjects Act and with the ethical standards on human experimentation in the Netherlands. The study was conducted in accordance with the Declaration of Helsinki,[18] approved by the medical ethics committee at the Maastricht University Medical Centre+ (MUMC+) (NL50072.068.14) and registered at clinicaltrial.gov (NCT02438618). CONSORT guidelines were followed (Table S1). Monitoring was performed by the sponsor and TFS Develop (Zaltbommel, The Netherlands). The investigators had unrestricted access to all data.

Population

This study is part of a multi-centre randomised, controlled trial (RCT). The study protocol has previously been published.[6] Patients were recruited at the out-patient ENT department at MUMC+. The inclusion criteria were at least 18 years of age and found to be eligible for unilateral BAHS surgery. The exclusion criteria were: (I) a history of immunosuppressive disease, (II) use of systemic immunosuppressive medication, (III) bilateral BAHS placement, (IV) relevant dermatological disease (*e.g.* psoriasis, severe eczema), (V) participation in other studies and (VI) when no suitable site for a 4-mm wide implant was found during surgery. In addition, patients had to agree to voluntary biopsies during surgery and the 12-week follow-up. All patients provided written informed consent.

Procedures

Baseline characteristics including gender, age, body mass index, smoking habits, medical history and medication were obtained in the case report forms. Patients received a Ponto Wide implant with a mounted abutment (Oticon Medical AB, Askim, Sweden). Prior to incision, skin thickness was measured to determine the appropriate abutment length according to the surgical manual.[19,20] Surgical techniques included the linear incision technique with soft-tissue preservation[6,21] or the minimally invasive Ponto surgery (MIPS) technique.[6,22] An elaborate description is provided in the protocol publication.[6] In the control group, the linear incision technique with soft tissue preservation was performed. Here a retro-auricular incision is made. The incision is opened and a central area of periosteum is removed. The implant site is prepared using a guide drill and countersink drill. The BAHS is placed with 40-50 Ncm insertion torque setting. The incision is closed with dermal sutures. The abutment is recovered using a 5-mm skin punch. [6,19,21]

In the test group, the MIPS technique was performed. Skin and subcutaneous tissue is removed with a 5-mm punch. Remaining soft tissue is removed with a raspatorium. The MIPS-cannula is inserted and filled with saline. The implant site is prepared using the specifically designed guide drill and widening drill. After removal of the cannula the BAHS is placed with 40-50 Ncm insertion torque setting. An installation indicator is used to aid in the estimation of complete insertion.[6,20,23]

During surgery, bone quality was assessed by the surgeon while drilling as very soft, soft, medium, hard or very hard. Post-surgery, a healing cap with gauze drenched in ointment (Terra-cortril, Pfizer Laboratories, New York, USA) was placed on the abutment. Subjects were followed for regular follow-up visits at 9-days, 3 weeks, 12 weeks and 1 year post-surgery. At 9 days post-surgery, the healing cap was removed. If necessary, local ointment (Terra-cortril) was used to promote healing. Instructions for aftercare were provided to all subjects. All subjects received an aftercare kit provided by the implant manufacturer (Oticon Medical AB). Subjects were instructed to clean the skin around their abutment each day using plain water and a soft toothbrush. 3 weeks post-

surgery, the implant was loaded with a BAHS sound processor (Ponto Plus or Ponto Plus Power, Oticon Medical AB).

The skin-penetrating abutment and peri-abutment skin were assessed at regular follow-up visits and during extra consultations. The Holgers Index grading was used: "0 No irritation; 1 Slight redness; 2 Red and slightly moist tissue, no granuloma formation; 3 Reddish and moist, sometimes granulation tissue; 4 Removal of skin-penetrating implant necessary due to infection".[5] Pain scores indicated by the patient, with 0 indicating no pain at all and 10 indicating the worst conceivable pain, were obtained on all visits.

A 5-mm skin punch located at the implant site, which is removed during surgery, was collected for baseline gene expression. At the twelve-week follow-up, a voluntary 1 mm biopsy (Integra, York, USA) was obtained after infiltration with local anaesthetics. During cases of soft-tissue inflammation, defined as a Holgers Index of ≥ 2, between surgery and the one-year follow-up, an additional biopsy was obtained. All the samples were snap frozen in liquid nitrogen and stored at -80°C. High-resolution photographs (NIKON D800E, NIKON CORPORATION, Tokyo, Japan) with an additional lens (Nikon AF-S VR Micro-NIKKOR 105 mm f/2.8G IF-ED, NIKON CORPORATION, Tokyo, Japan) were obtained prior to each biopsy (surgery, 12-week follow-up and cases of inflammation).

RNA extraction and quantitative real-time polymerase chain reaction

RNA was isolated from the frozen samples using TRI Reagent (Sigma, St. Louis, MO, USA), according to the manufacturer's protocol. The RNA concentration was measured with the DeNovix DS-11 spectrophotometer. 750ng of RNA was used to transcribe to cDNA using the SensiFast cDNA Synthesis Kit, according to the manufacturer's protocol. Primers were obtained for genes related to inflammation (IL-1β, IL-6, IL-8, TNF-α, IL-17, IL-10, TGF-ß, MIP-1α), tissue metabolism (MMP-9, TIMP-1, COL1α1), vascularisation (VEGF-A, FGF-2) and bacterial infection (TLR-2, TLR-4) (Table S2) (Sigma-Aldrich, St. Louis, Missouri, United States). PCR experiments were performed by a dedicated technician and reported following MIQE guidelines.[24] To quantify the mRNA expression levels, a qRT-PCR analysis was performed with a LightCycler480 (Roche) using a three-step PCR program. Relative gene expression levels were derived using the LinRegPCR (version 2016.1) method and normalised to the geometric average of two reference genes, cyclophylin A (CyloA) and beta-2-microglobulin (β2M). Non-detectable samples were imputed as half the lowest observed threshold. Fold changes were calculated using the delta-delta CT method.

Statistical analysis

Statistical analyses were performed using R version 3.3.2 (R Foundation for Statistical Computing, Austria). Statistical significance was established at $p \leq 0.05$. Gene mRNA expression is presented for all genes. Due to the small sample size and non-normality of the data, non-parametric tests were executed. Differences between baseline and the 12-week follow-up were compared using Wilcoxon's signed rank test. Differences between surgical techniques were compared at baseline (pre-surgery) and post-implantation at the 12-week follow-up using the Mann-Whitney U test. Samples taken during episodes of inflammation were compared with mRNA expression at 12 weeks using Wilcoxon's signed rank test. The post-implantation 12-week follow-up expression of patients who did not experience an episode of inflammation and the post-implantation 12-week follow-up expression of patients who did experience an episode of inflammation during follow-up were compared using the Mann-Whitney U test.

Twelve-week expression of the group of patients without episodes of inflammation from 12 weeks and onwards, was compared with patients experiencing episodes of inflammation at any time during the follow-up using the Mann-Whitney U test.

Pre-surgical mRNA (baseline) expression was correlated to bone quality, smoking status, diabetes and body mass index (BMI) using Spearman's rank-order correlation test. Post-surgical mRNA expression was correlated to pain scores, Holgers Index 0 vs 1 scores, smoking status, the presence of diabetes and BMI using Spearman's rank-order correlation test.

Results

Population

Thirty-five of the total of 49 patients participating in the randomised, controlled trial, running from December 2014 to July 2016, agreed to the voluntary 12-week biopsy. Biopsies from 22 patients had cDNA of sufficient quality to be processed. Seven patients experienced at least one episode of inflammation and five of them agreed to an additional biopsy. Patient characteristics are summarized in Table 1. Ten patients were fitted with a BAHS using the MIPS technique and 12 patients were fitted with a BAHS using the linear incision technique with soft-tissue preservation. Skin thickness and abutment length used per surgical group are presented in Table 1. Soft-tissue outcomes at follow-up are presented in Table 2.

Table 1: Characteristics

Age (years)		51.68 (24.44)
Gender		
Male		7 (32%)
Female		15 (68%)
Body mass index (kg/m2)		29.27 (6.80)
Smoking		
Non-smoker		16 (73%)
Smoker		6 (27%)
Diabetes		3 (14%)
Skin thickness (mm)		6.5 (1.9)
Surgical technique		
MIPS		10 (45%)
Linear incision with soft-tissue preservation		12 (55%)
	MIPS (n=10)	Linear incision technique (n=12)
Skin thickness (mm)^	6.5	6
Abutment length		
9 mm	5	7
12 mm	3	4
14 mm	2	1

For continuous variables, the mean (standard deviation) is presented. For categorical variables, the number (%) is presented. ^ median is presented.

Table 2: Skin outcomes

Holgers Index scores at 12 weeks	
Holgers Index 0	12 (55%)
Holgers Index 1	10 (45%)
Holgers Index >1	0 (0%)
Additional biopsy during inflammation	5 (23%)

Numbers (%) are presented.

Post-implantation expression

The expression of mRNA for the selected cytokines at baseline and the 12-week follow-up is shown in Figure 1 and Figure S1. The results for mRNA gene expression at 12 weeks post-implantation compared with baseline and differences between surgical techniques are presented in Table 3. The variation in mRNA expression at baseline for the different cytokines was high.

The analysis revealed that, 12 weeks post-implantation, a significantly higher expression of the inflammatory markers, IL-1β and IL-8, was observed compared with baseline for both surgical techniques, whereas the IL-6 mRNA expression was significantly down-regulated. Tissue metabolism markers, MMP9, TIMP-1 and COL1α1, were significantly up-regulated 12 weeks post-implantation. The vascular endothelial growth factor, VEGF, displayed a trend toward increased mRNA expression at 12 weeks. Post-implantation, the mRNA expression of basic fibroblast growth factor, FGF-2, was significantly decreased. Compared with baseline, no significant differences were observed for the bacterial marker, TLR-2.

Table 3: The influence of implantation and surgical technique on cytokine mRNA expression

	Post-implantation		Surgical technique		
Gene	Fold change	p-value	MIPS technique	Linear incision technique	p-value
IL-1β	16.3 (134.5)	0.002*	$1.2*10^{-3}$ ($1.6*10^{-3}$)	$2.9*10^{-3}$ ($2.4*10^{-2}$)	*0.06*
IL-6	$7.3*10^{-2}$ (0.7)	0.009*	$2.6*10^{-3}$ ($1.3*10^{-2}$)	$7.1*10^{-3}$ ($8.8*10^{-3}$)	0.92
IL-8	2.0 (18.9)	0.03*	$1.0*10^{-12}$ ($1.3*10^{-3}$)	$4.1*10^{-4}$ ($5.7*10^{-3}$)	*0.06*
TNF-α	0.5 (2.9)	0.341	$9.0*10^{-4}$ ($2.2*20^{-3}$)	$2.8*10^{-3}$ ($3.9*10^{-3}$)	0.20
IL-17	1.1 (25.0)	0.234	$1.0*10^{-12}$ ($1.5*10^{-5}$)	$1.0*10^{-12}$ ($8.3*10^{-5}$)	0.84
TGF-ß	1.2 (2.7)	0.95	$1.4*10^{-2}$ ($1.7*10^{-2}$)	$1.2*10^{-2}$ ($1.8*10^{-2}$)	0.95
MIP-1α	0.2 (4.1)	0.799	$7.4*10^{-4}$ ($3.0*10^{-3}$)	$2.9*10^{-3}$ ($9.2*10^{-3}$)	0.16
MMP-9	18.5 (71.5)	0.005*	$1.0*10^{-12}$ ($1.2*10^{-4}$)	$8.1*10^{-4}$ ($7.8*10^{-3}$)	0.11
TIMP-1	1.5 (5.7)	0.006*	$2.1*10^{-2}$ ($2.0*10^{-2}$)	$2.1*10^{-2}$ ($2.8*10^{-2}$)	1.0
COL1α1	1.3 (12.9)	<0.001*	0.27 (0.60)	0.42 (0.73)	0.58
FGF-2	0.2 (0.4)	0.004*	$1.9*10^{-2}$ ($5.1*10^{-2}$)	$3.0*10^{-2}$ ($4.0*10^{-2}$)	0.64
VEGF	0.9(3.8)	*0,095*	$2.2*10^{-2}$ ($2.6*10^{-2}$)	$1.7*10^{-2}$ ($1.0*10^{-2}$)	0.90
TLR2	0.3 (3.1)	0.198	$1.3*10^{-2}$ ($4.9*10^{-2}$)	$3.3*10^{-2}$ ($4.5*10^{-2}$)	0.20

Post-implantation: the median (interquartile ranges) is presented for fold change post-implantation compared with baseline. p-values of relative mRNA expression post-implantation at 12-week follow-up compared with baseline (pre-surgery) using Wilcoxon's signed rank test are presented. Surgical technique: the median (interquartile ranges) is presented for relative mRNA expression per surgical technique at 12-week follow-up. p-values of relative mRNA expression at 12-week follow-up comparing the linear incision with soft-tissue preservation surgical technique with the MIPS technique using the Mann-Whitney U test are presented. * indicates p-value ≤ 0.05

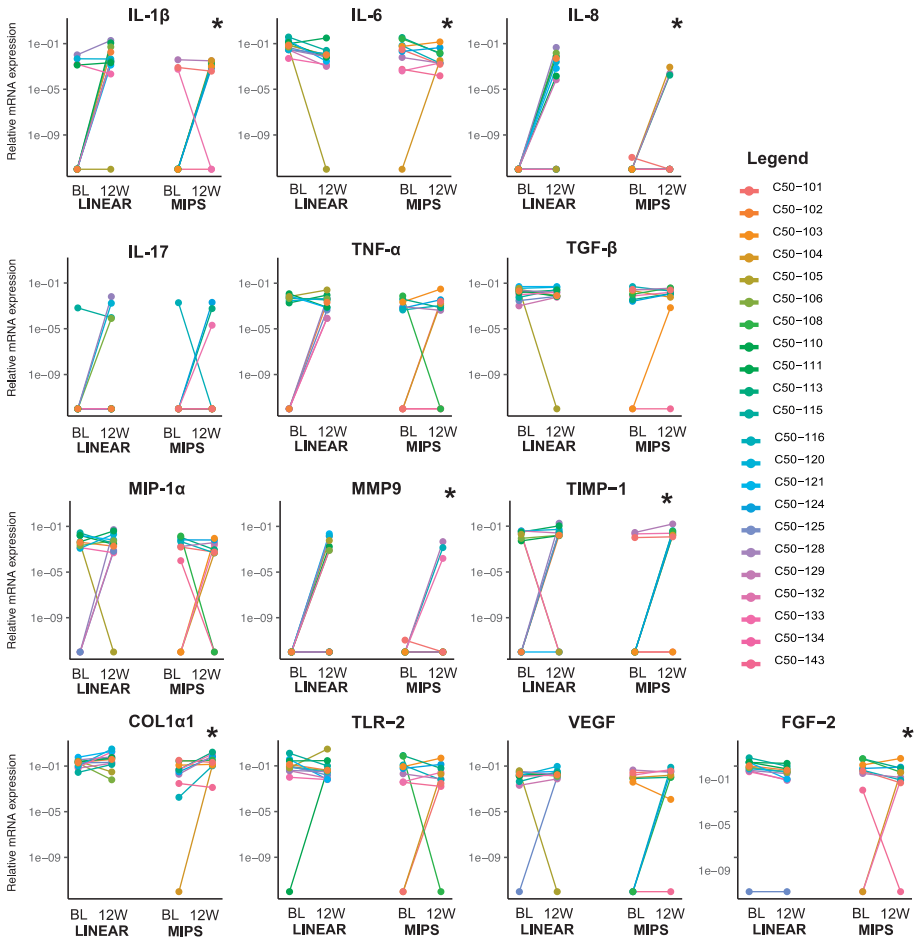


Figure 1. Relative mRNA expression within patients at baseline and 12-week follow-up**.** BL indicates pre-surgical baseline mRNA expression. 12w indicates mRNA expression post-implantation at 12-week follow-up visit. LINEAR indicates the linear incision technique with soft-tissue preservation. MIPS indicates the minimally invasive Ponto surgery technique. Non-detectable mRNA expression measurements are not shown. In most samples, the mRNA expression of IL-10 and TLR4 was not detectable and it is therefore not shown. * indicates p-value ≤ 0.05.

Surgical technique

The comparative analysis between the two different surgical techniques revealed no significant difference in gene expression in any of the genes at 12 weeks (Table 3). In spite of this, trends towards a higher expression of IL-8 and IL-1β were found in the linear incision group compared with the MIPS group (Figure 2/Table 3). No significant differences in gene expression were observed between the groups at baseline (Table S3).

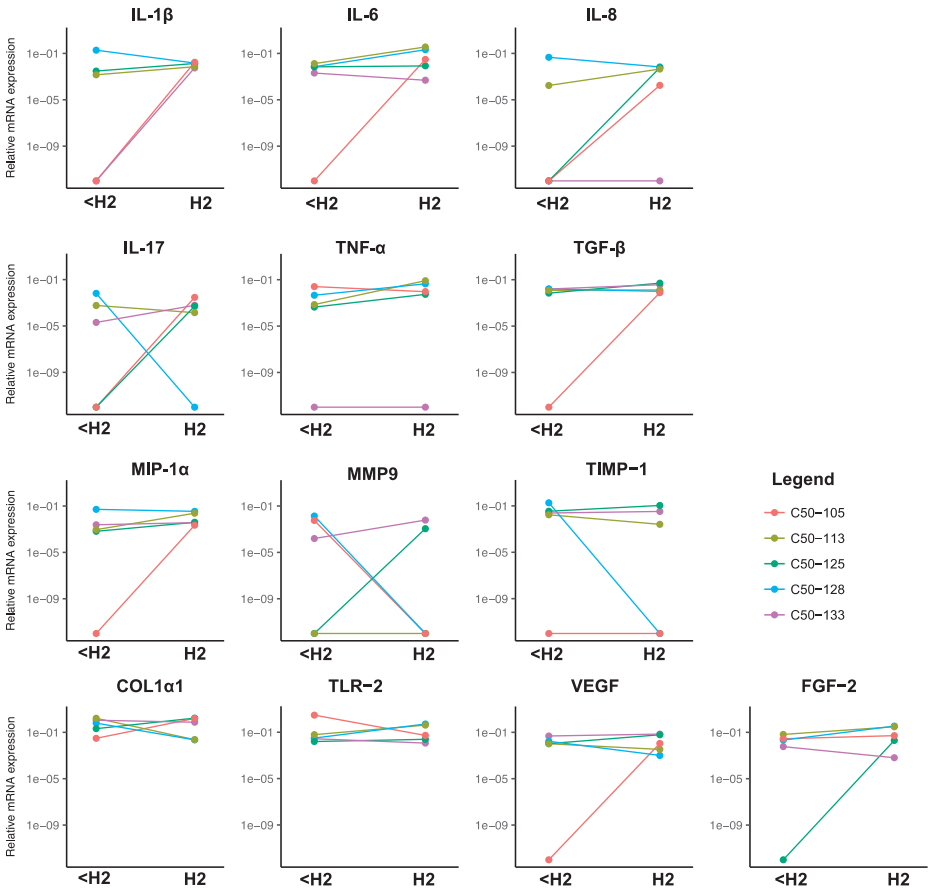


Figure 2: Relative mRNA expression within subjects between 12-week follow-up and episodes of inflammation. *< H2* indicates relative expression post-implantation at 12-week follow-up with Holgers Index 0-1 scores. *H2* indicates relative expression during cases of inflammation (Holgers Index 2 scores).

Inflammation

Seven patients experienced an episode of inflammation between 12-week follow and one year follow-up. Five of them agreed to an additional biopsy. The mRNA expression levels at the time of inflammation are presented in Figure 2. The gene expression analysis revealed that, during an episode of inflammation, the pro-inflammatory cytokines, IL-1ß and IL-17, were significantly up-regulated compared with patients without an episode of inflammation during follow-up (Table 4, Between patients; Inflammation/Figure S2). For the inflammation samples at the episode of inflammation, TLR-2, which is related to bacterial recognition, and the inflammatory mediator, TNF-α, displayed a trend towards higher mRNA expression compared with samples from patients without inflammation. In contrast, in patients experiencing inflammation, no differences in mRNA expression were observed at the time of inflammation compared with the non-inflamed state at 12 weeks (Table 4, Within patients, Inflammation). Similarly, there were no differences in gene expression at 12 weeks post-implantation between the 15 patients who did not experience an episode of inflammation during follow-up compared with the seven patients who did (Table 4, Between patients; Post-implantation).

Table 4: Cytokine mRNA expression during inflammation within and between patients

	Within patients		Between patients	
Gene	Fold change	Inflammation^	Post-implantation	Inflammation#
IL-1β	62.3 (15741)	0.188	0.83	0.015*
IL-6	0.9 (3.3)	0.188	0.78	0.23
IL-8	0.02 (0.06)	0.855	1.0	0.16
TNF-α	15.3 (38)	0.361	0.32	*0.05*
IL-17	6.2 (468)	0.813	0.15	0.02*
TGF-ß	3.7 (6.5)	0.188	0.11	0.80
MIP-1α	15.4 (24)	0.438	0.57	*0.10*
MMP-9	17.5 (997)	0.855	0.73	0.96
TIMP-1	1.5 (2.2)	0.855	0.72	0.54
COL1α1	4.6 (11)	1.0	0.73	1.0
FGF-2	2.7 (149)	0.125	0.86	0.35
VEGF	0.3 (234)	0.438	0.097	0.62
TLR2	2.4 (7.2)	1.0	*0.07*	*0.07*

Median (interquartile ranges) fold changes for relative mRNA expression during inflammation compared with baseline are presented. * indicates p-value ≤ 0.05.

^Within-subject inflammation: post-implantation mRNA expression at 12 weeks (n=5) is compared with mRNA expression post-implantation during inflammation (n=5) using Wilcoxon's signed rank test.

Between-subject post-implantation (n=22): post-implantation 12-week mRNA expression for patients who did not experience inflammation during follow-up (n=15) is compared with post-implantation 12-week mRNA expression for subjects who experienced an episode of inflammation during follow-up (n=7) using the Mann-Whitney U test.

% Between-subject Inflammation: mRNA expression during cases of inflammation (n=5) is compared with post-implantation 12-week mRNA expression for subjects who did not experience an episode of inflammation during follow-up (n=15).

Clinical parameters

Multiple exploratory correlations were performed between gene expression and the clinical parameters. Significant correlations with a correlation coefficient of 0.6 or higher are presented in Table 5. The full correlation data sets are included as Supporting Information (Tables S4 and S5). Holgers Index 1 compared with Holgers Index 0 scores showed a strong negative correlation to the mRNA expression of the pro-inflammatory cytokine TNF-α mRNA expression, indicating that Holgers 0 is associated with a higher TNF-α expression. A strong positive correlation was found for smoking status and post-implantation MMP-9 expression, related to tissue metabolism, as well as to extracellular matrix degradation. In addition, a moderate positive correlation was found for post-implantation IL-8 mRNA expression and smoking status. Baseline bone quality was moderately associated with a higher mRNA expression of the inflammatory cytokine, IL-1β, and the vascularisation marker, VEGF (Table S4). Further, VEGF mRNA expression at the 12-week follow-up was moderately negatively correlated to the presence of diabetes (Table S5).

Table 5: Exploratory correlation analyses for mRNA expression and clinical parameters

	Baseline			12 weeks		
Clinical parameter	Gene	rs	p-value	Gene	rs	p-value
Holgers Index 0 vs. 1						
				TNF-α	-0.75	<0.001
Smoking						
				IL-8	0.60	0.003
				MMP-9	0.85	0.04

mRNA expression for moderate to strong statistically significant positive or negative correlations with different clinical parameters analysed in the patients is presented. The complete output data can be found in Supporting Information Tables S2, S3 and S4.

Discussion

Summary

To our knowledge, this is the first prospective study to investigate the molecular profile related to inflammation after BAHS implantation. Skin reactions and the local inflammatory gene expression of patients with and without clinical signs of inflammation were compared within and between patients. In addition, the influence of surgical technique was evaluated. The results reveal that, three months after the implantation of a BAHS, there is

an up-regulation of genes related to inflammation (IL-1β, IL-8) and tissue remodelling (COL1α1, MMP-9 and TIMP-1) within the soft tissue close to the abutment. In contrast, IL-6 and FGF-2 decreased significantly. During inflammation (Holgers Index ≥ 2), the mRNA expression of IL-1β, TNF-α, IL-17 and TLR-2 increased. Strong, moderate and weak correlations were found for the post-implantation mRNA expression of various genes and bone quality, smoking status or presence of diabetes. No significant differences were found between the surgical techniques with respect to gene expression in the soft-tissue biopsies.

BAHS implantation

Our results indicate that a continuous state of increased immune activation is present within the soft tissue surrounding the abutment, despite a lack of macroscopic signs of inflammation. This is in line with previous microscopic observations.[14] In addition, the relatively higher expression of tissue anabolic (COL1α1) and catabolic (MMP-9 and TIMP-1) genes at 12 weeks compared with baseline suggests an ongoing remodelling process in the skin surrounding the abutment at this point after implantation. We observed a relatively large variation in mRNA expression between individuals at baseline and post-implantation. Our results did not show a relation between this variation and future inflammation. We believe that, by presenting individual data per patient, a more accurate presentation of variability is provided, allowing for the visual inspection and interpretation of the complex interplay between the biomaterial and tissue, surgical technique, inflammation and immune response.

In consonance with the previous BAHS study and dental studies, we found that several inflammation and tissue remodelling related genes were upregulated. [17,25] Strikingly, IL-6 and FGF-2 showed a significant downregulation in mRNA expression. Depending on the micro-environment IL-6 can have an pro-inflammatory or anti-inflamatory properties with a large inter-individual variability. [26] Immune responses in peri-implant tissue are impaired.[27–29] In some, but not all subjects reduced expression of IL-6 was observed at 12-week follow-up. The decreased expression of IL-6 might be explained as a direct or indirect reduced immune response. Secondly, in the skin next to the abutment non-keratinized epithelial down growth has been observed. [30] These changes in skin composition may possibly lead to differences in immune responses. Alternatively, at baseline a full skin thickness biopsy is obtained and the 12-week follow-up a 1-mm biopsy is less deep possibly related differences in expression.

Inflammation and the Holgers index

The Holgers Index is routinely used as one of the major endpoints in BAHS studies.[5,31] It captures external signs of inflammation, such as moistness or redness. In agreement with the observations of Grant *et al.,* we found that IL-1β, IL-17, MIP-1α, TNF-α and TLR-2 may correlate to inflammation.[17] In contrast, for Holgers 1 scores, we found negative correlations for IL-1β, TNF-α and TLR-2 mRNA expression. These contrasting results may indicate

that the Holgers Index may not necessarily be correlated to gene expression as a continuous scale. The Holgers Index was mainly designed for skin reduction techniques. With the shift towards skin preservations techniques, a relatively large area of the soft-tissue column facing the abutment is not observed. Bearing in mind that the only difference between 0 and 1 is "slight redness", the full soft-tissue column facing the abutment may be an important factor which is omitted using the Holgers Index for skin preservation techniques. Moreover, the difference between Holgers 0 and Holger 1 scores may only reflect between-subject variations instead of clinically relevant differences requiring treatment, such as the prescription of topical or oral antibiotics.[7] Pooling available datasets and studies assessing the inter- and intra-rater reliability of the Holgers Index may help in identifying the biological validity and reliability of rating scales for peri-abutment dermatitis.

Surgical technique

MIPS was designed on the assumption that reduced surgical trauma would lead to improved outcomes.[32] Due to the less invasive nature of MIPS, we hypothesised that immune responses might be less pronounced. The inflammatory cytokines, IL-1β and IL-8, display trends towards lower expression in MIPS compared with the linear incision technique, which may be a confirmation of the hypothesis. Mueller *et al.* observed less inflammation and improved healing outcomes for flapless approaches for dental implants.[33,34] The limited sample size and quality of cDNA obtained for BAHS warrant further study regarding the influence of surgical technique.

Clinical parameters

Smoking, BMI and diabetes have all been postulated as factors for soft-tissue reactions or implant loss in BAHS and dental studies.[35–37] Recently, Sayardoust *et al.* showed that smoking is associated with different gene profiles post-surgery for dental implants.[38] Here, we observed that, with intact skin, no gene expression differences were observed. However, when the skin integrity is impaired by the BAHS, smoking status increases the expression of genes related to inflammation and tissue remodelling indicating a possible biological relationship for this risk factor. Strong to moderate correlations were found for IL-8 and MMP-9 expression. BMI and diabetes might affect the gene expression as well. However, only moderate to weak correlations were found here (Table S5). We found no correlation between pain scores and gene expression for BAHS.

Strengths and limitations

This is the first prospective study to evaluate cytokine expression within the soft tissue around the abutment in BAHS. The influence of both installing the device and inflammation was investigated within and between patients. We were able to correlate cytokine

expression to several clinical parameters. On the other hand, this study also has several limitations. This study is a tertiary endpoint of a larger randomized controlled trial comparing surgical techniques. For this sub-study, no formal sample size calculation was executed. Ideally, this study would have been conducted in a larger set-up with more time points. The resulting sample size is limited, thereby restricting us to create models that include several factors or performing more elaborate factor analyses. Although, cytokine expression will vary from 12 weeks and onwards we assumed that for the overall group expression is relatively stable in order to compare 12-week expression for subjects without inflammation to the cases of inflammation. In the comparative analysis, we would have preferred to execute all the qRT-PCR reactions in triplicate. However, the cDNA yield was too low to do this and several patients were excluded due to cDNA yields that were too low. We investigated mRNA expression, which may differ from protein expression. However, to limit this possibility, we used protein coding mRNA transcripts. The location of sampling was close to the BAHS, but it may have differed from patient to patient, due to manipulation and movements. At follow-up, several patients mentioned that they believed they had experienced more complaints such as crust formation or episodes of inflammation after the 12-week biopsy. By obtaining a biopsy in the area close to the abutment, a possible source of infection may have been created. In future studies, the use of the periostrip paper as a less invasive alternative for collecting the peri-abutment fluid should be considered.[17] A non-invasive method might increase willingness to provide samples, thereby increasing the sample size and enabling the collection of samples at several time points. In dental implants, bone quality is usually assessed using the classification introduced by Lekholm and Zarb [39]. This qualification includes a radiographic assessment combined with an tactile assessment performed during drilling. Unfortunately, no such qualification exists for BAHS. Also, radiological assessment of the future implant location is rare. Therefore, in this study we used an explorative bone quality assessment scale based on tactile feedback only.

Perspective

Implant-associated inflammation and infections represent a challenge in BAHS and other medical devices.[29] New submerged active and passive bone conduction devices that result in intact skin have been introduced in the last few years.[40,41] Even though they seem like good alternatives, they may provide too low amplification for some patients. Moreover, these solutions require more invasive surgery, are probably more expensive and have relatively large imaging artefacts of at least 9-10 cm for MRI investigations.[42] Another approach would be to adjust the surface properties of the percutaneous abutment. Abutments coated with hydroxyapatite that are believed to integrate with the skin have been introduced.[43] An alternative approach using extra-smooth abutment surfaces is currently being investigated (NCT02304692). Antibiotic-releasing, steroid-releasing and silver-coated abutments might be possible as well. It is hypothesised that the introduction of

skin preservation techniques will help the immune system surrounding the abutment to remain intact, thereby improving immune responses. In addition, the use of punch-only techniques could reduce skin movements. Immune modulation, abutment properties, surgical damage and reduced skin movements may all contribute to the occurrence of inflammation. The combination of bacterial data, cytokine expression, skin movements and long-term follow-up data may provide additional insights into implantation and inflammation. [6] Here, we found that implantation itself may already result in a state of continued inflammation.

Conclusion

The results of the present randomised, prospective clinical trial show that, after 12 weeks of BAHS implantation, the gene expression of some inflammatory cytokines (IL-8 and IL-1β) is still relatively high compared with the baseline, steady-state, expression. The up-regulation of anabolic (COL1α1) and tissue-remodelling (MMP-9 and TIMP1) genes indicates an ongoing remodelling process after 12 weeks of implantation. The results suggest that IL-1β, IL-17 and TNF-α may be interesting markers associated with inflammation.

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Supplementary material

Table S2: Primers

Generic name	Forward primer (5' → 3')	Reverse primer (5' → 3')
IL-1β (interleukin 1, beta)	CTGAGCTCGCCAGTGAAATG	TGTCCATGGCCACAACAACT
IL-6 (interleukin 6)	ACCCCCAATAAATATAGGACTGGA	TTCTCTTTCGTTCCCGGTGG
IL-8 (interleukin-8 (IL-8)/ CXCL8	CCACCGGAAGGAACCATCTC	TTCCTTGGGGTCCAGACAGA
TNF-α (tumour necrosis factor)	CTGGGCAGGTCTACTTTGGG	CTGGAGGCCCCAGTTTGAAT
IL-17 (interleukin 17)	AACCGATCCACCTCACCTTG	TCTCTTGCTGGATGGGGACA
IL-10 (interleukin 10)	ACATCAAGGCGCATGTGAAC	TAGAGTCGCCACCCTGATGT
TGF-ß (transforming growth factor beta)	GGGCTACCATGCCAACTTCT	GACACAGAGATCCGCAGTCC
MIP-1α (CCL3 C-C motif chemokine ligand 3)	TCGAGCCCACATTCCGTCAC	GCAGCAAGTGATGCAGAGAAC
MMP-9 (matrix metallopeptidase 9)	CATCCGGCACCTCTATGGTC	CATCGTCCACCGGACTCAAA
TIMP-1 (metallopeptidase inhibitor 1)	CATCCGGTTCGTCTACACCC	TCTGCAGTTTGCAGGGGATG
COL1α1 (collagen, type I, alpha 1)	TGCTCGTGGAAATGATGGTG	CCTCGCTTTCCTTCCTCTCC
FGF-2 (fibroblast growth factor 2)	CCACCTATAATTGGTCAAAGTGGT	TCATCAGTTACCAGCTCCCC
VEGF-A (vascular endothelial growth factor A)	CTGTCTAATGCCCTGGAGCC	ACGCGAGTCTGTGTTTTTGC
TLR2 (toll-like receptor 2)	GTGTTGCAAGCAGGATCCAA	GCAGTGAAAGAGCAATGGGC
TLR4 (toll-like receptor 4)	GAATGCTAAGGTTGCCGCTT	TTAGGAACCACCTCCACGC
Beta-2-microglobulin (β2M)	TCCATCCGACATTGAAGTTG	CGGCAGGCATACTCATCTT
Cyclophylin A (CyloA)	CTCGAATAAGTTTGACTTGTGTTT	CTAGGCATGGGAGGGAACA

Table S3: Cytokine expression at baseline

Gene	Surgical technique		
	MIPS technique	Linear incision technique	
IL-1β	$1.0*10^{-12}$ ($4.3*10^{-4}$)	$1.0*10^{-12}$ ($1.4*10^{-3}$)	0.66
IL-6	$2.5*10^{-2}$ ($6.3*10^{-2}$)	$6.0*10^{-2}$ ($7.5*10^{-2}$)	0.16
IL-8	$1.0*10^{-12}$ (0)	$1.0*10^{-12}$ (0)	0.32
TNF-α	$5.6*10^{-4}$ ($1.9*10^{-3}$)	$2.4*10^{-3}$ ($5.5*10^{-3}$)	0.43
IL-17	$1.0*10^{-12}$ (0)	$1.0*10^{-12}$ (0)	0.90
IL-10	NA	NA	NA
TGF-ß	$9.1*10^{-3}$ ($2.2*10^{-2}$)	$2.1*10^{-2}$ ($2.2*10^{-2}$)	0.28
MIP-1α	$1.6*10^{-3}$ ($6.1*10^{-3}$)	$2.7*10^{-3}$ ($6.3*10^{-3}$)	0.69
MMP-9	$1.0*10^{-12}$ (0)	$1.0*10^{-12}$ (0)	0.32
TIMP-1	$1.0*10^{-12}$ ($7.5*10^{-3}$)	$6.9*10^{-3}$ ($2.5*10^{-2}$)	0.19
COL1α1	$3.9*10^{-2}$ ($9.3*10^{-2}$)	0.19(0.15)	0.41
FGF-2	$4.0*10^{-2}$ (0.1)	$7.6*10^{-2}$ (0.17)	0.31
VEGF	$6.0*10^{-3}$ ($1.4*10^{-2}$)	$1.6*10^{-2}$ ($1.6*10^{-2}$)	0.12
TLR2	$4.0*10^{-2}$ (0.1)	$8.2*10^{-2}$ (0.13)	0.34
TLR4	NA	NA	NA

Median relative expression (interquartile ranges) is presented for relative mRNA expression per surgical technique at baseline. p-values of relative mRNA expression at 12-week follow-up comparing the linear incision with soft-tissue preservation surgical technique with the MIPS technique using the Mann-Whitney U test are presented * indicates p-value ≤ 0.05. NA: not applicable

Table S4: Correlation analysis for baseline cytokine expression

Gene	Bone quality		Smoking		Diabetes		BMI	
	r_s	p-value	r_s	p-value	r_s	p-value	r_s	p-value
IL-1β	0.51	0.02*	-0.21	0.34	-0.27	0.23	-0.06	0.80
IL-6	0.02	0.93	0.16	0.47	0.03	0.89	-0.12	0.58
IL-8	0.26	0.25	-0.15	0.51	-0.09	0.70	-0.05	0.82
TNF-α	-0.04	0.87	0.15	0.50	0.12	0.60	-0.21	0.35
IL-17	-0.22	0.33	-0.22	0.33	-0.13	0.58	0.14	0.54
TGF-ß	0.23	0.31	0.26	0.24	-0.17	0.46	-0.05	0.82
MIP-1α	0.10	0.65	-0.07	0.76	0.07	0.74	-0.13	0.57
MMP-9	0.26	0.25	-0.15	0.51	-0.09	0.70	-0.05	0.82
TIMP-1	0.10	0.66	0.01	0.97	-0.13	0.58	-0.07	0.76
COL1α1	0.31	0.16	0.16	0.47	-0.14	0.55	-0.20	0.37
FGF-2	0.01	0.96	0.18	0.43	0.07	0.75	-0.28	0.21
VEGF	0.53	0.01*	0.22	0.27	-0.03	0.89	-0.19	0.39
TLR2	-0.15	0.51	0.22	0.32	0.03	0.89	0.08	0.73

* indicates p-value ≤ 0.05. A positive correlation for bone quality indicates harder bone as assessed during surgery.

Table S5: Correlation analysis for 12-week follow-up cytokine expression

Gene	Pain scores		Bone quality		Diabetes		BMI		Holgers 0 vs Holgers 1		Smoking	
	r_s	p-value	r_s	p-value	r_s	p-value	r_s	p-value	r_s	p-value	r_s	p-value
IL-1β	-0.01	0.96	-0.1	0.96	-0.45	0.04*	-0.14	0.54	-0.47	0.03*	0.43	0.03*
IL-6	0.06	0.77	-0.7	0.77	-0.32	0.14	-0.32	0.15	-0.32	0.15	0.42	0.05*
IL-8	-0.26	0.25	0.21	0.35	-0.19	0.40	-0.25	0.26	-24	0.29	0.60	0.003*
TNF-α	0.01	0.95	0.00	>0.99	-0.07	0.75	-0.31	0.16	-0.75	<0.001*	0.49	0.02*
IL-17	0.11	0.63	*0.40*	*0.06*	0.01	0.96	0.17	0.46	-0.10	0.65	-0.07	0.77
TGF-ß	0.04	0.88	0.12	0.61	-0.43	<0.05*	-0.04	0.88	0.06	0.79	0.04	0.87
MIP-1α	-0.31	0.16	0.20	0.37	-0.43	<0.05*	*-0.36*	*0.10*	-0.33	0.13	0.45	0.03*
MMP-9	-0.04	0.86	0.30	0.18	-0.06	0.80	0.01	0.95	-0.08	0.74	0.85	0.04*
TIMP-1	0.04	0.85	-0.16	0.49	-0.37	0.09	*0.39*	*0.07*	-0.29	0.20	-0.13	0.56
COL1α1	0.01	0.96	0.43	0.05*	-0.24	0.28	-0.02	0.95	0.27	0.23	0.02	0.92
FGF-2	0.05	0.82	0.01	0.97	-0.06	0.78	-0.44	0.04*	-0.37	0.09	*0.41*	*0.06*
VEGF	-0.18	0.41	0.26	0.25	-0.54	0.01	-0.04	0.86	-0.26	0.24	0.08	0.71
TLR2	0.10	0.67	0.54	0.14	0.14	0.55	-0.16	0.48	-0.47	0.03*	0.27	0.23

* indicates p-value ≤ 0.05. A positive correlation for bone quality indicates harder bone as assessed during surgery.

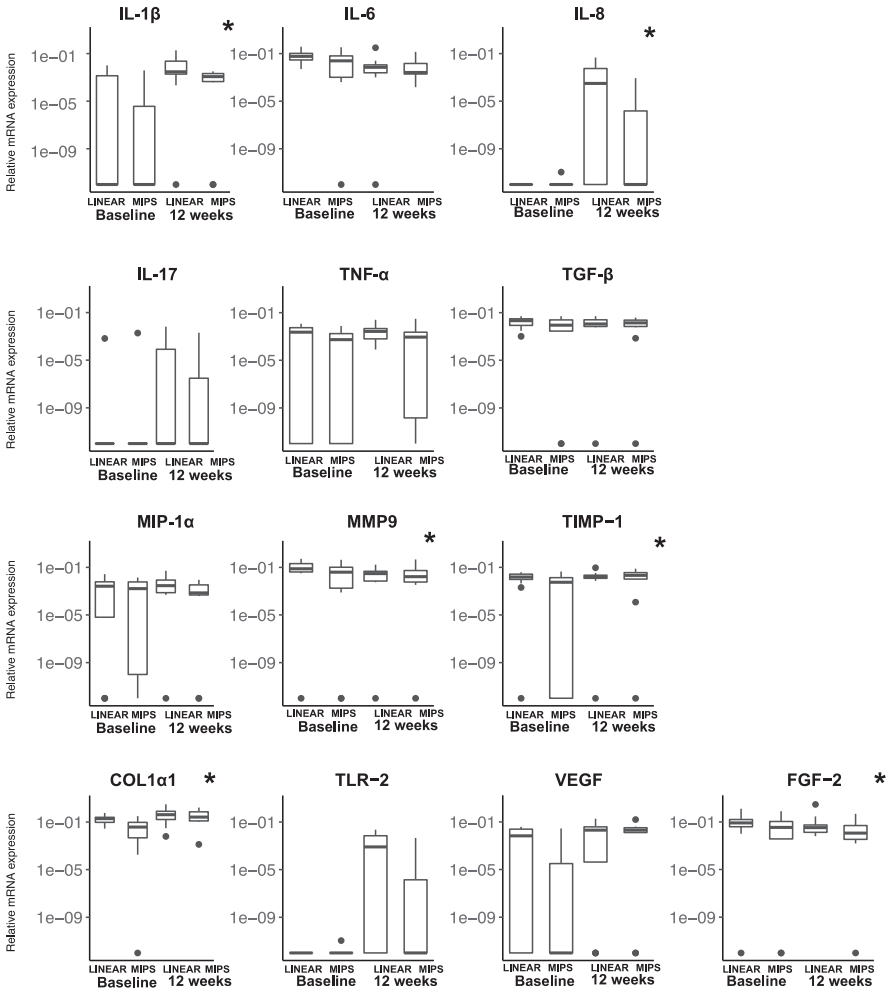


Figure S1: Relative mRNA expression at baseline and 12-week follow-up *LINEAR* indicates the linear incision technique with soft-tissue preservation. *MIPS* indicates the minimally invasive Ponto surgery technique.

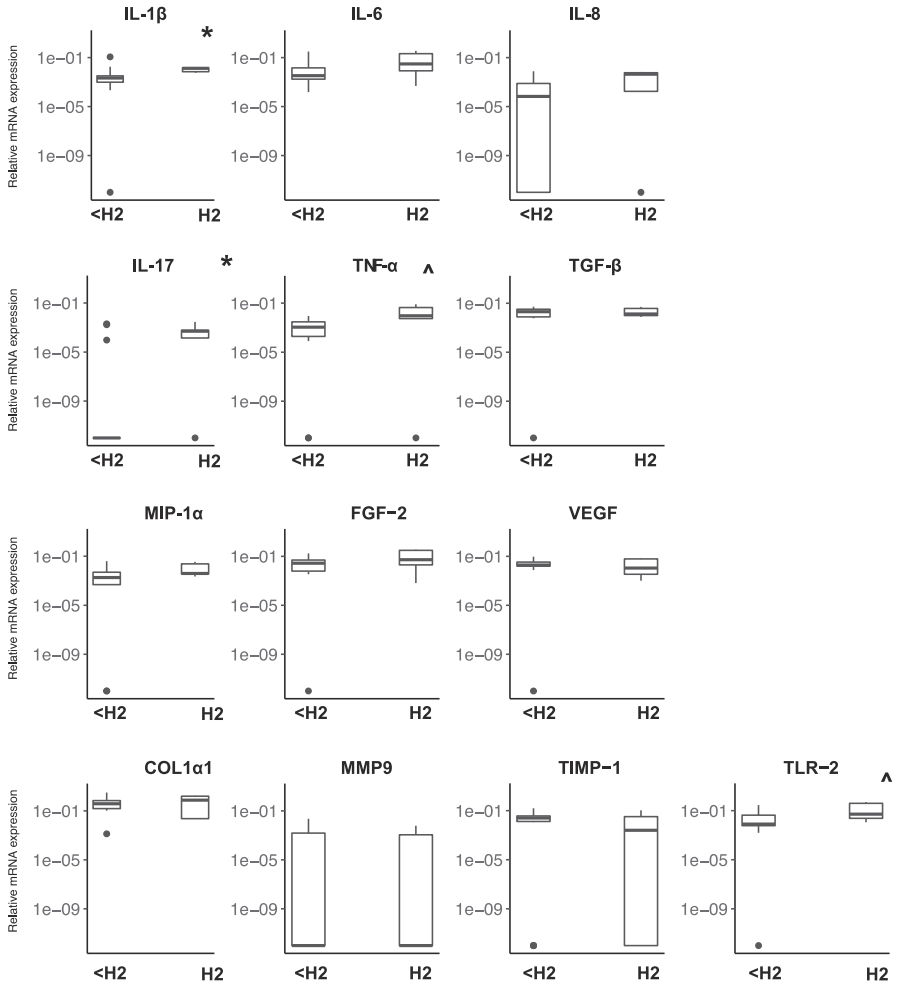
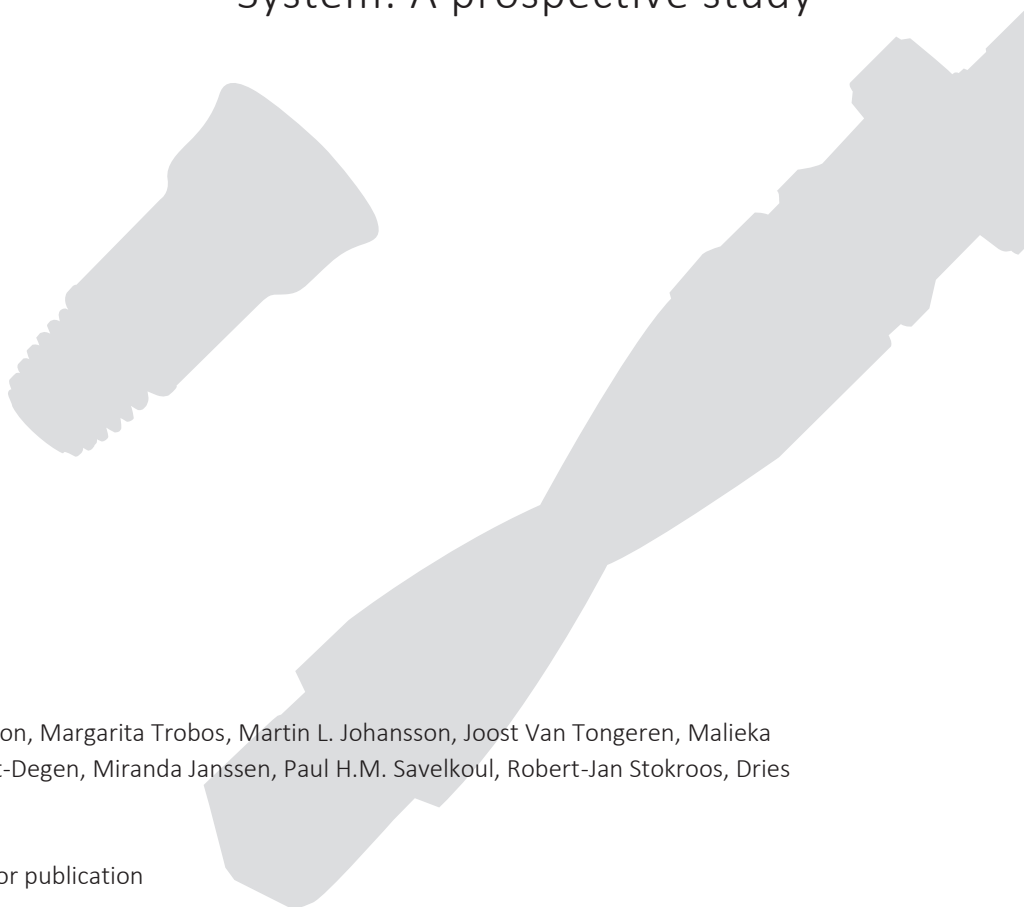


Figure S2: Relative mRNA expression post-implantation at 12-week follow-up and during episodes of inflammation between subjects < indicates relative expression post-implantation at 12-week follow-up with Holgers Index 0-1 scores. H2 indicates relative expression during cases of inflammation (Holgers Index 2 scores). * indicates p-value ≤ 0.05. ^ indicates p-values between 0.05-0.10.

Chapter 7

Microbiome on the Bone-Anchored Hearing System: A prospective study



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Submitted for publication

Abstract

The percutaneous bone-anchored hearing system (BAHS) has evolved to a common treatment option for various types of hearing revalidation. Soft tissue reactions are a common complication associated with BAHS and are generally poorly understood. This study aims to investigate the influence of BAHS and associated skin reactions on the peri-abutment skin microbiome. A total of 45 patients were prospectively followed from implantation up to at least one year. Swabs were obtained at baseline, 12 weeks follow-up and during cases of inflammation (Holgers score ≥2). The microbiota was assessed using IS-pro™, a bacterial profiling method based on the interspace region between the 16S-23S rRNA genes. Detection of operational taxonomic units, Shannon Diversity Index, sample similarity analyses and Partial Least Squares Discriminant Analysis (PLS-DA) were employed. *Staphylococcus epidermidis, Streptococcus pneumoniae/mitis, Propionibacterium acnes, Staphylococcus capitis, Staphylococcus hominis, Bifidobacterium longum, Heamophilus parainfluenzae, Lactobacillus rhamosus, Bordetella* spp., *Streptococcus sanguinis, Peptostreptococcus anaerobius, Staphylococcus aureus, Lactococcus lactis, Enterobacter cloacae* and *Citrobacter koseri* were the most commonly found bacterial species. *Streptococcus pneumoniae/mitis* was significantly more often observed after implantation, whereas *Propionibacterium acnes* was significantly less observed in the inflamed group than the non-inflamed. The relative abundance of *Staphylococcus epidermidis* (17%) and *Staphylococcus aureus* (19.4%) was the highest for the group of patients with inflammation. The Shannon Diversity Index was significantly increased after implantation compared with pre-surgical swabs for Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia (FAFV), but not for other phylae or other bacteria. The diversity index was similar post-surgically for patients experiencing inflammation and for patients without inflammation. With a supervised classifier (PLS-DA), patients prone to inflammation could be identified at baseline with an accuracy of 91.7%. In addition, PLS-DA could classify post-surgical abutments as non-inflamed or inflamed with an accuracy of 97.7%.

This study shows the potential of using IS-pro™ to describe and quantify the microbiome associated with the percutaneous BAHS. The skin microbiome associated with a present or future skin inflammation could be identified. Both *S. aureus* and *S. epidermidis* should be considered as relevant bacteria for BAHS-associated inflammation.

Introduction

In 1977, the bone-anchored hearing system (BAHS) was introduced. The BAHS is a retro-auricular titanium implant that is fixed in the skull via osseointegration. A skin-penetrating abutment is placed on the implant allowing the coupling of a sound processor (Tjellstrom et al., 1981). The BAHS is considered a successful treatment option with overall good outcomes. It is an established therapy for patients suffering from several types of hearing loss including conductive hearing loss, mixed hearing loss and single sided deafness (Snik et al., 2005; Faber et al., 2015). During the last years, treatment options have become less invasive, resulting in improved outcomes regarding aesthetics, pain, numbness of the skin, implant survival and soft tissue reactions (Hultcrantz, 2013; den Besten et al., 2016; Calon et al., 2018). Soft tissue reactions, such as inflammation of the peri-abutment skin, are still a common complication, but the majority of cases are relatively easily treated (Dun et al., 2012; Verheij et al., 2016).

Soft tissue reactions can reduce overall patient satisfaction, processor use and possibly quality of life. Additionally, patients require extra consultations in the out-patient clinic. Implant-associated infections represent a challenge for BAHS as well as for other medical devices (Busscher et al., 2012). The peri-abutment skin surrounding the BAHS is usually graded according to the Holgers Index, consisting of a five-point grading scale (Holgers et al., 1988). Several factors have been postulated to influence the aetiology of adverse soft tissue reactions and inflammation including; implant design, strains and stresses in the peri-abutment tissue, surgical technique, immune responses, patient related factors and biofilm formation (Monksfield et al., 2011; Johansson, 2018; Trobos et al., 2018). Most likely, a complex interplay between these factors exists.

The skin next to the abutment does not attach to the abutment, instead there is an epithelial down growth with non-keratinized tissue (Holgers et al., 1995a). Post-BAHS surgery, the anatomical situation is different from that of normal skin due to the permanent breach of the skin. This specific skin-abutment transition zone could constitute a special niche containing a distinct set of bacterial flora that differs from the normal skin (Trobos et al., 2018).

In relation to BAHS, several bacteria have been detected and bacterial colonisation of implants and abutments have been shown (Holgers et al., 1995b; Monksfield et al., 2011; Trobos et al., 2018). *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS)*, Bacteroides urealyticus, Proteus, Klebsiella, Escherichia coli and Peptostreptococcus* have been cultured from non-inflamed and inflamed BAHS (Holgers and Ljungh, 1999). *Staphylococcus aureus* and *Staphylococcus epidermidis* can infect cells adjacent to the implant surface (Boelens et al., 2000; Broekhuizen et al., 2008b; Garzoni and Kelley, 2009). Moreover, peri-implant bacteria may even re-colonize an implant after antibiotic treatment, becoming a source of infection (Broekhuizen et al., 2008a).

Biofilms have been found on both non-inflamed and inflamed BAHS (Monksfield et al., 2011; van Hoof et al., 2015). Biofilms consist of layers of bacterial cells and their

secreted extracellular polymeric substances, and are particularly resistant to antibiotics (Busscher et al., 2012) . Recently, Buskermolen *et al.* showed that oral biofilms could be classified as either supporting oral wound healing or related to pathogenic responses (Buskermolen et al., 2018). It is conceivable that similar mechanisms could play a role in soft tissue reactions in BAHS.

Bacteria, whether as single, part of biofilm or intra-cellular, are all sources of potential infection related to implants. Insight into the microbiome profiles and changes related to BAHS implantation and skin inflammation could increase the knowledge and understanding of the role of the microbiome in BAHS. This knowledge could be used to prevent skin inflammation or improve treatment directed at specific pathogens.

In this study, we prospectively evaluated the microbiome profiles and its changes by using IS-pro™, a molecular technique based on profiling of the bacterial 16S-23S ribosomal interspace region (Budding et al., 2010). This study aims to (I) evaluate the bacterial changes induced by BAHS surgery and (II) to identify a microbiome profile associated with peri-abutment skin inflammation.

Methods and Materials

Ethics

This study is part of a larger trial comparing two surgical techniques for installing BAHS (Calon et al., 2016) and the short-term clinical results have recently been published (Calon et al., 2018). This study was performed in accordance with the Dutch legislation of Medical Research Involving Human Patients Act and with the ethical standards on human experimentation in the Netherlands. The study was conducted in accordance with the Declaration of Helsinki and was approved by the medical ethical committee of Maastricht University Medical Centre + (MUMC+) (NL50072.068.14) and registered at clinicaltrial.gov (NCT02438618). Consent procedure was in accordance with the study protocol and ISO 14155.

Population

Patients were recruited at the out-patient department of otorhinolaryngology of MUMC+. All patients were enrolled in a randomized controlled clinical trial comparing two surgical BAHS techniques. Patients had to be older than 18 years and qualify to undergo unilateral BAHS surgery. Exclusion criteria were: (I) a history of immunosuppressive disease, (II) usage of systemic immunosuppressive medication, (III) bilateral BAHS placement, (IV) relevant dermatological disease (*e.g.* psoriasis, severe eczema), (V) participation in other studies, and (VI) when no suitable site for a 4-mm Ponto Wide implant was found during surgery. All patients provided written informed consent. Complete clinical data, including additional patients not participating in the swab collection have been

published previously (Calon et al., 2018). This paper reports the subset of patients where swab samples were collected.

Procedures

Baseline characteristics including sex, age, body mass index, smoking habits, medical history and medication were recorded in the Case Report Form. Patients received a Ponto Wide implant with a pre-mounted abutment (Oticon Medical AB, Askim Sweden) using single stage surgery. Both the linear incision technique with soft tissue preservation (Hultcrantz, 2011; Calon et al., 2016) and the Minimally Invasive Ponto Surgery (MIPS) technique were used for BAHS implantation surgery (Calon et al., 2016; Johansson et al., 2017). For nine days following surgery, a healing cap and gauze drenched in antibiotic ointment (Terra-Cortril, Pfizer, New York, USA) was applied on the abutment.

Patients were followed for standard follow-up visits at 9 days, 3 weeks, 12 weeks and 1 year. Prior to surgery, samples for bacterial analyses were obtained from (i) the intended implantation site (baseline implant skin sample, BIS) and (ii) contra-lateral control side (baseline control sample, BCS). Before BIS was taken, the hair was shaven. No disinfectants were used. A swab was used to obtain a sample from an area of 2 by 2 cm at the intended implant site. The BCS was obtained at the retro-auricular area without any prior cleaning or shaving. A swab was used to obtain a sample from an area of 2 by 2 cm. After the BIS was obtained, the skin was cleaned with antiseptics before surgery was commenced.

During follow-up, swab samples were obtained from the peri-abutment skin site (peri-abutment sample, PAS) and contra-lateral control site (contra-lateral sample, CLS) at (i) 12 weeks and (ii) during episodes of inflammation. The PAS was obtained by swabbing the external side of the abutment 360 degrees. Thereafter, the same swab was immediately used to swab approximately 1 cm of peri-abutment skin 360 degrees around the abutment. If peri-abutment fluid (*e.g.* moist) was present at the peri-abutment skin, this was obtained as well. No cleaning was performed before the sample was obtained. The CLS was obtained in a similar manner as the BCS where 2 by 2 cm of unshaved skin was swabbed at the contralateral side of the abutment without prior cleaning and disinfection.

For BAHS, the Holgers Index is used to assess the peri-abutment skin. It is a five-grade scale where; 0 No irritation; 1 Slight redness; 2 Red and slightly moist tissue, no granuloma formation; 3 Reddish and moist; sometimes granulation tissue; 4 Removal of skin-penetrating implant necessary due to infection (Holgers et al., 1988). In the context of this study, peri-abutment skin inflammation was considered present if the patient were rated of having a Holgers Index score of two or higher. If the patient demonstrated peri-abutment skin-inflammation (Holgers Index score ≥2), either at a standard follow-up visit or an extra consultation, extra swabs were taken at the inflamed peri-abutment site (iPAS) and the contra-lateral site (CLS). Samples were stored in an Eppendorf container with 200 µL Transportbuffer (IS-Diagnostics, Amsterdam) at -20°C.

For DNA extraction an easyMAG machine (bioMérieux Clinical Diagnostics, Marcy l'Etoile, France), an automated system for total nucleic acid isolation, was used. To every sample, 500 µL of nucliSENS lysis buffer was added. This suspension was shaken for 5 min at ≥1400 rpm and subsequently centrifuged at 18000 g for 2 min. The complete volume was transferred to an 8-welled easyMAG container and 2 mL nucliSENS lysis buffer was added. After incubation at room temperature for ≥10 min, 70 µL of magnetic silica beads were added. Afterwards, the mixture was inserted in the easyMAG machine and the "specific A" protocol was chosen, selecting the off-board workflow and eluting DNA in 70 µL of buffer. All extracted DNA was stored at 4°C.

IS-pro™ profiling of microbiota

Isolated DNA was further processed as recommended by the manufacturer according to the IS-pro™ assay (IS-Diagnostics, Amsterdam, The Netherlands). The IS-pro™ procedures have been previously published (Budding et al., 2010; Rutten et al., 2015; de Meij et al., 2016). In short: the procedure consists of two standardized PCR amplifications. The first PCR is specific for Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia (FAFV) and Bacteroidetes. FAFV includes many skin bacteria. The second PCR is specific for Proteobacteria. Amplifications were carried out on a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA). Cycling conditions for PCR were: 10 cycles at 94°C for 30 s, 67°C (1°C decrease per cycle) for 45 s, and 72°C for 1 min; followed by 25 cycles at 94°C for 30 s, 57°C for 45 s, and 72°C for 1 min; and the final step at 72°C for 11 min and cooling down to 4°C. A total of 5 µL of PCR amplification product was mixed with 20 µL of IS-pro™ eMix (IS-Diagnostics). PCR fragment analysis was performed on an ABI Prism 3500 genetic analyser (Applied Biosystems).

Data analysis and statistics

Data were analysed using the proprietary IS-pro™ software (IS-Diagnostics, Amsterdam, The Netherlands) and R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was assumed at $p \leq 0.05$. Data was visualized in Spotfire version 7.10 (TIBCO, Palo Alto, CA, USA). Species were identified according to IS-pro™ fragment profiles, with the use of IS-pro™ software. Heatmaps were created by hierarchical clustering of samples by the unweighted pair group method with arithmetic mean (UPGMA).

Prevalence of species

Prevalence of the most common bacterial species on BIS and PAS at 12-weeks were compared using McNemar's test. Samples obtained from non-inflamed sites (PAS) were compared with samples obtained from cases with inflammation (iPAS) using McNemar's test. For patients with several episodes of inflammation, only the first swab obtained during the first episode of inflammation was used in the analysis.

Sample similarity

Log2 transformed microbiome profiles were compared pairwise within and between patients with cosine correlation coefficients for all bacteria and FAFV (Daniels et al., 2014; de Meij et al., 2016). Mean (*M*) and Standard Deviation (*SD*) were calculated.

Sample diversity

To determine bacterial diversity within-patients, Shannon index results were computed for all bacteria. Shannon diversity index changes overtime within-patients were evaluated using paired sample t-test. Between subject comparisons (no inflammation vs. inflammation) were evaluated using an independent samples t-test. To correct for multiple testing the Bonferroni method was used for the number of phyla tested. All distributions were checked for normality. In case of non-normality, the non-parametric Wilcoxon signed-rank test or Mann-Whitney U test was performed.

Partial least square discriminant analysis

Partial least squares discriminant analysis (PLS-DA) was used to classify swabs for clinical status. PLS-DA entails an algorithm-based classification method designed to identify Operational Taxonomic Units (OTU) as predictors for predefined classifications (Perez-Enciso and Tenenhaus, 2003; Rajilic-Stojanovic et al., 2011; Daniels et al., 2014). Classifications included inflammation status for PAS (PAS vs iPAS) and future inflammation during follow-up for BIS. The Variable Importance for Projection (VIP) criterion was used to identify OTUs that discriminate between groups. A VIP score > 1.2 was considered as the relevant threshold.

Results

Patient characteristics

From December 2014 to January 2017, 49 patients were included. Four patients were excluded due to implant loss during follow-up. For one patient, the baseline swab was missing, and this patient was therefore excluded from analyses involving baseline implantation site swabs. Patient characteristics are summarized in Table 1. Mean age was 52 years (*SD*=15). Mean Body Mass Index was 28 kg/m^2 (*SD*=6). Ten patients were smokers (22%) and 35 non-smokers (78%). A total of 22 (49%) patients received a BAHS using the MIPS procedure and 23 (51%) patients received a BAHS using the linear incision technique with soft tissue preservation. During the study, 12 (27%) patients experienced at least one episode of inflammation.

Table 1: Patient characteristics

Characteristics	(n=45)
Age (years)	52 (15)
Gender	
Male	18 (40%)
Female	27 (60%)
Body mass index	28 (6)
Diabetes	6 (13%)
Smoking	
Non-smoking	35 (78%)
Smoking	10 (22%)
Surgical technique	
MIPS	22 (49%)
Linear incision with soft tissue preservation	23 (51%)
At least one episode of inflammation	12 (27%)
At least two episodes of inflammation	5 (11%)

Mean (*SD*) is presented for continuous variables. Number (%) is presented for categorical variables.

Species

An overview of the ten most commonly found bacteria on the BIS, PAS (Holgers score ≤1) and iPAS (inflamed peri-abutment skin/Holgers Index score ≥ 2) is presented in Figure 1A. *Staphylococcus epidermidis* (found in 86.4%-97.8% of patients) followed by *Streptococcus pneumoniae/mitis* (found in 55%-80% of patients) were the two most commonly observed species for all three types of samples. *Propionibacterium acnes* (61.4%), and the two CoNS species *Staphylococcus capitis* (47.7%) and *Staphylococcus hominis* (45.5%) were commonly observed on the BIS. Similarly, but in less proportion, *S. hominis* (40%), *Haemophilus parainfluenza* (40%), and *P. acnes* (35.6%) were commonly found on non-inflamed PAS. During inflammation, the peri-abutment site (iPAS) was frequently colonized by *S. capitis* (45%), *Bifidobacterium longum* (40%), *S. hominis* (35%), *S. aureus* (30%) and *Streptococcus sanguinis* (30%). McNemar's test revealed that the PAS had significantly more *Streptococcus pneumoniae/mitis* (p=0.03) and significantly less *Propionibacterium acnes* (p=0.02) compared with the BIS. McNemar's test between PAS and iPAS demonstrated no significant differences for the amount of *S. aureus* (p=0.62), *P. acnes* (p=1.0,) or any of the other bacterial species within subjects.

The relative abundance of *S. aureus* was 19.4% for the patients with inflammation (iPAS) compared with 6.1% for the patients without inflammation PAS (Figure 1B). However, post-hoc, Wilcoxon signed-rank tests revealed no significant differences in relative abundance for *S. aureus* (p=0.21) or any of the other species when comparing non-inflamed PAS with inflamed PAS.

Although *S. epidermidis* (17%) was the second most abundant species in cases of inflammation, a similar proportion was detected in non-inflamed cases and at baseline.

Other bacteria such as *S. hominis* and *S. pneumoniae/mitis* showed a lower contribution to the complete bacterial load observed on the abutment during cases of inflammation.

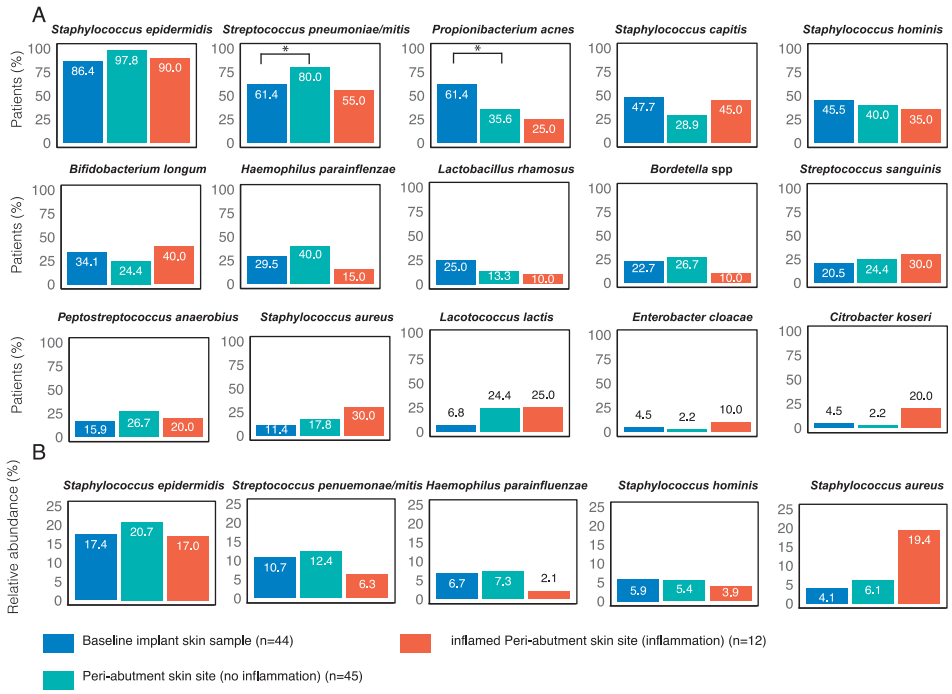

Figure 1: Prevalence of the most commonly found bacterial species associated with BAHS.
Sample sites: Baseline implant skin (BIS), non-inflamed peri-abutment skin (PAS, Holgers Index score <2) and inflamed peri-abutment skin (iPAS, Holgers Index score ≥2). (A) The percentage of patients with observed bacterial species is presented. (B) Relative abundance (in percentage) of five commonly found species based on the total bacterial counts detected in the three sample types: baseline implant skin sample (BIS), peri-abutment skin site (PAS) and inflamed PAS (iPAS). *indicates p-value ≤ 0.05.

Diversity analysis

The UPGMA clustered heat map comparing BIS to PAS is presented in Figure 2. No clear distinction between 12-week PAS and BIS could be observed. Although one 12-week PAS cluster was observed, BIS and 12-week PAS for the same patient generally did not cluster together. Shannon diversity index for the BIS, PAS, iPAS and CLS is presented in Figure 3. For all bacteria combined, there was no significant difference in Shannon diversity index between PAS at 12 weeks and BIS (p=0.16). However, the PAS at 12-week follow-up showed a significantly higher diversity index for bacteria from the FAFV group compared with the BIS (p=0.04). Within subject analyses showed no significant differences for Proteobacteria and Bacteroidetes between BIS and PAS at 12-weeks. The comparative

analyses between PAS and iPAS showed no significant differences for FAFV, Proteobacteria or Bacteroidetes.

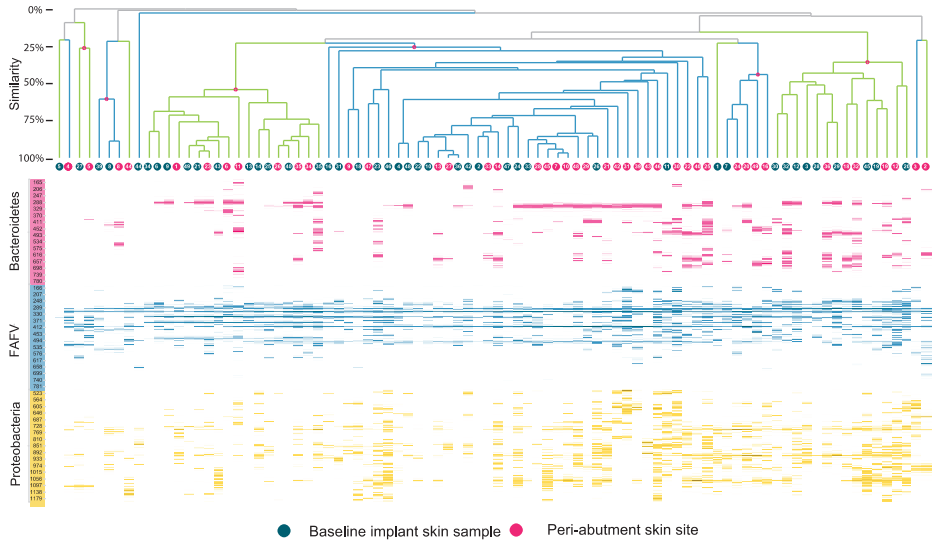

Figure 2: Clustered heatmap with IS-pro profiles for baseline implant skin samples and peri-abutment skin sites obtained at 12-week follow-up. Red dots and branches indicate the percentage of similarity between swabs below. Swabs are clustered per phylum. FAFV represents the phylae Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia. Patient identification numbers are indicated in dots. All baseline implant skin samples and all 12-week peri-abutment skin site swabs are included. Overall no specific pattern can be observed distinguishing baseline implant skin samples and peri-abutment skin sites, neither any intra-patient cluster.

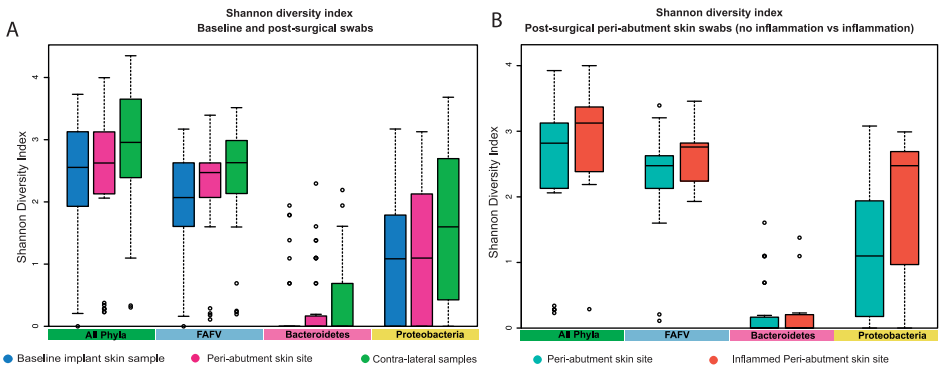

Figure 3: Shannon diversity index. A: Shannon diversity index for baseline implant skin samples, 12-week peri-abutment skin site swabs, and contra-lateral samples obtained at 12-week follow-up. B: Shannon diversity index for non-inflamed peri-abutment skin swabs at 12 weeks and inflamed peri-abutment skin swabs obtained during follow-up.

Sample similarity

All similarity results are presented in Table 2. At baseline, the similarity within patients was 59% for all bacteria combined and 77% for FAFV, and between patients the similarity was lower with 43% and 60%, respectively. Baseline implant skin and peri-abutment skin samples showed higher similarities within patients for all bacteria combined (45%) and FAFV (62%) compared to between subject similarities for all bacteria (38%) and FAFV (55%). Small differences were observed between PAS and iPAS within subject similarities.

Table 2: Sample similarities

Percentage of sample similarities	All bacteria (SD)	FAFV (SD)
Baseline		
Similarity between baseline implant skin sample (BIS) and contra-lateral samples (n=10)		
Intra-subject	59 (21)	77 (14)
Inter-subject	43 (15)	60 (12)
12-week follow-up		
Similarity between peri-abutment skin site (PAS) and baseline implant skin sample (BIS) (n=44)		
Intra-subject	45 (13)	62 (16)
Inter-subject	38 (11)	55 (13)
Peri-abutment skin site (intra-subject)		
Similarity between peri-abutment skin site (PAS, iPAS) and baseline implant skin sample (BIS)		
No inflammation (n=32)	46 (0.13)	63 (15)
Inflammation (n=12)	44 (0.19)	62 (15)
Similarity between peri-abutment skin site (PAS, iPAS) and contra-lateral samples (CLS)		
No inflammation (n=32)	48 (0.13)	68 (13)
Inflammation (n=12)	57 (19)	70 (19)

Similarity coefficients (standard deviations) obtained from Log2 data are presented.

Partial least square discriminant analysis

Results for the PLS-DA analyses are presented in Figure 4. The first PLS-DA analysis aimed to predict patients prone to inflammation. For all the 32 patients not experiencing inflammation during the follow-up period, all 32 BIS swabs were classified as no inflammation in the future. For the 12 patients experiencing inflammation, 9 of the 12 BIS swabs were correctly classified in the group experiencing inflammation in the future. Nine out of 12 baseline swabs were correctly classified in the group experiencing inflammation in the future. This yielded a sensitivity of 75%, specificity of 100% and accuracy of 93.3% for BIS classified as prone to inflammation in the future. The second PLS-DA analysis aimed to identify iPAS. Thirty-three cases of non-inflamed PAS and 11 of 12 cases of iPAS were

correctly classified as non-inflamed and inflamed respectively resulting in a sensitivity of 91.7%, specificity of 100% and accuracy of 97.7%.

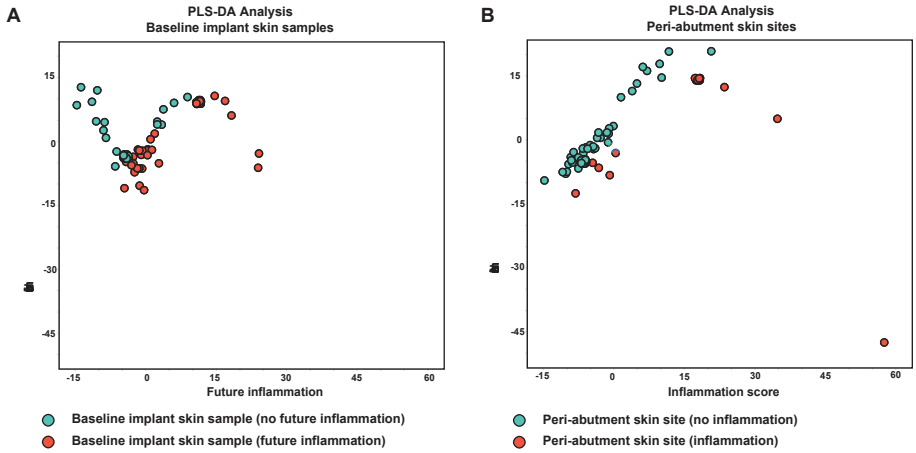

Figure 4: Partial least square discriminant analysis. A) Analysis of baseline implant skin samples with no inflammation (green) during the 1-year follow-up. Red dots indicate baseline implant skin samples (BIS) with inflammation during follow-up. B) Analysis of peri-abutment skin site swabs (PAS) obtained at 12-week follow-up with no inflammation during follow-up (green dots) and peri-abutment skin site swabs obtained during inflammation (iPAS) (red dots). Inflammation was defined as a Holgers Index score ≥2. On the x-axis the first component, (future) inflammation, in the PLS-DA model is displayed. On the y-axis the second component, no (future) inflammation, is displayed.

Discussion

Species

In this prospective study, BAHS recipients were followed for at least one year. To date, the influence of implantation of the skin penetrating BAHS on the skin microbiome has remained elusive. By employing a validated molecular based profiling technique, we could provide an overview of the microbiome for the skin of the retro-auricular crease and the peri-abutment skin, both under normal conditions and in cases of inflammation.

Previous work has mainly evaluated the microbiology of BAHS using conventional culture techniques (Holgers and Ljungh, 1999; Trobos et al., 2018). In line with our results, Holgers scorings of 0 and 1 have been shown to be mainly associated with CoNS, *P. acnes* and *S. aureus (Holgers and Ljungh, 1999)*. During healthy and inflammatory conditions, CoNS (specially *S. epidermidis*) and *S. aureus* have been cultured (Holgers et al., 1992; Holgers and Ljungh, 1999; Trobos et al., 2018) and their presence was further confirmed in this study using a molecular method. Therefore, *S. aureus* and *S. epidermidis* should be considered as relevant bacteria associated with BAHS. In fact, *S. epidermidis*, as part of the native flora of the skin, may be introduced to the site when the skin is breached by

the abutment, explaining its increased presence at the site where the abutment penetrates the skin. Furthermore, *S. epidermidis* ability to form biofilms enables it to adhere to medical devices and be protected from the patient's immune response and antibiotics (Mack et al., 1996). This could also have contributed to the increased presence of *S. epidermidis* on and around the abutment compared with baseline.

During cases of peri-abutment inflammation, high counts of *S. aureus*, *S. epidermidis*, and *Streptococcus pneumoniae/mitis* were isolated, with *S. aureus* as the most abundant bacterium. Especially compared with baseline and non-inflamed PAS, the relative presence of *S. aureus* on iPAS was striking. Interestingly, we observed a decrease in *S. epidermidis* and an increase of *S. aureus* during inflammation. Using PLS-DA analysis, it was possible to classify patients prone to inflammation and inflamed abutments with a high accuracy. Therefore, the IS-pro™ technique could have clinical usefulness in the early detection of susceptible patients to inflammation and serve as a tool for the follow-up of patients at risk for soft tissue complications.

Correlations and diversity

At baseline, the similarity analysis between implant site swabs and skin controls for all bacteria was higher within patients than between patients, meaning a similar microbiome was present on the skin of both retro-auricular creases within each subject. Furthermore, at baseline a strong similarity was found within patients for FAFV. This indicates that within patients the gram-positive fraction of the microbiome on both sides of the retro-auricular head strongly correlates. Based on descriptive statistics, the correlation results indicate that the microbiome from samples obtained over time is more similar within patients than between patients in this study group. This observation has been previously reported using 16S rRNA metagenomic sequencing showing less intrapersonal variation in microbiome between symmetrical skin sites than the interpersonal variation (Gao et al., 2007; Costello et al., 2009; Grice et al., 2009). It has been suggested that external environmental factors (climate and geography), host factors (immune status and pathophysiology) and historical exposures may account for the interpersonal variation (Grice and Segre, 2011).

In addition, within patients, moderate to strong correlations of FAFV between the peri-abutment skin and the baseline implant skin as well as the baseline control sample was demonstrated. In contrast, a weaker correlation was observed between patients. Differences in cosine correlations between non-inflamed PAS and iPAS were small with only 1-2%. Our results could indicate that within subjects the microbiome is relatively stable. Within the limitations of our study, both a similar normal skin microbiome and slightly less similar abutment microbiome seem to exist between individuals. Moreover, the Shannon Diversity index significantly increased for skin bacteria after BAHS implantation. These results are in line with previous observations, indicating that diversity of skin bacteria increases after BAHS implantation (Trobos et al., 2018).

Peri-abutment skin reactions

In clinical practice, patients after receiving a BAHS, visit the outpatient clinic only once or twice after implantation and for 5-year check-up. Annual check-up varies per clinic. Adverse peri-abutment soft tissue reactions are reasons for extra consultations. Overall, patients that experience only one episode of inflammation can easily be treated with counseling regarding hygiene and local antibiotic treatment. However, patients recurring soft tissue complaints are clinically challenging and associated with higher costs and morbidity (Dun et al., 2012; Verheij et al., 2016). Treatment of these cases typically involves prescription of oral antibiotics but may also require temporary abutment removal or extensive tissue revision surgery. If these individuals could be identified prior to implantation surgery, preventive measures may be employed or alternative rehabilitation treatments without a skin penetrating abutment could be considered, including softband, patch and transcutaneous solutions. The sample size of only two patients experiencing recurrent episodes of infection limits the possibility to identify a specific microbiome for this clinically relevant population. In our practice the broad-spectrum antibiotic ointment Terra-Cortril, containing hydrocortisone, oxytetracycline and polymyxin-B, is often prescribed for soft tissue reactions around BAHS.

However, due to its limited effect on staphylococci, in case of inflammation, it could be advisable to subscribe different ointments such as Mupirocin (Bactroban, GlaxoSmithKline, Brentford, London, United Kingdom) or Fucidin ointment that target *S. aureus* and *S. epidermidis* (EUCAST, 2018). When considering therapies targeted towards skin infection, they might not only require the inhibition of pathogenic bacteria, but at the same time to promote the growth of symbiotic bacteria (Grice et al., 2009). Alternative approaches such as probiotics could be considered for future research.

Sample location

Recently, Trobos *et al.* demonstrated colonization of bacteria on the abutment, in the surrounding skin and in the peri-abutment fluid space (Trobos et al., 2018). The microbiological profiles in the soft-tissue close to the abutment, in the space between the abutment and tissue, and on the abutment over time yielded different total counts of selected bacterial groups. Results from the surrounding skin and peri-abutment space correlated most strongly with clinical outcome (Trobos et al., 2018). The microbiome profiles obtained in the current study may have been different if other sample types than swabs would have been included or if sampling would have been taken at specific compartments. With the technique used here, the swab touched the exterior part of the abutment, the skin surrounding the abutment and possibly the peri-abutment fluid.

Strengths and limitations of the study

This is the first prospective study investigating the effect of implantation and inflammation on the microbiome in association with BAHS using a molecular approach. The sample size is large considering this research field. The study design allowed the possibility to create a prediction model for patients prone to inflammation. For bacterial profiling, we employed IS-pro™ which is a validated 16S-23S Interspacer PCR-based profiling method. IS-pro™ uses 16S-23S region length in combination with specific polymorphisms to identify species per phylum. Phyla include FAFV, Bacteroidetes and Proteobacteria. This method allows for a fast analysis (<5 hours) of bacteria from different human samples(Budding, 2016).

Unidentified bacteria were observed in this study of which the clinical significance has yet to be determined. The database used to classify species can allow for some misidentification of species. Markers for antibiotic sensitivity are not obtained using IS-pro™. Therefore, isolation of the causative pathogen and its susceptibility testing would still be needed to guide targeted treatment. In this study, only 12 patients experienced at least one episode of inflammation, which might explain the non-significant differences observed in inflamed and non-inflamed peri-abutment swabs. The supervised PLS-DA algorithm was able to identify most cases of inflamed peri-abutment swabs and baseline implant skin swabs that experienced a future inflammation. Due to the relative small sample size these results warrant caution and should be cross-validated in a larger dataset to test for robustness. The peri-abutment skin was assessed using the Holgers Index. However, its biological or inter- and intra-observer reliability has been questioned (Calon et al., 2016; Kruyt et al., 2017).

Conclusion

The hereby-applied methods provide a proof-of-principle that IS-pro™ was able to describe and quantify the BAHS-associated microbiome. Patients with risk for experiencing a future peri-abutment skin inflammation could potentially be identified prior to implantation using IS-pro™. This study shows that microbiome diversity increases post-BAHS surgery, and specially *Staphylococcus aureus* and *Staphylococcus epidermidis* should be considered as relevant bacteria after BAHS implantation. Better understanding of the skin microbiome will help to elucidate (i) the microbial interdependencies necessary to maintain healthy skin conditions around BAHS, (ii) how specific bacterial species are involved in BAHS-associated infection and inflammation, (iii) and facilitate the development of new antimicrobial strategies.

Acknowledgements

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Conflict of interest

MJ is employee of Oticon Medical. PS and AEB are co-owners of IS-Diagnostics (Amsterdam, The Netherlands). The other authors declare no conflict of interest.

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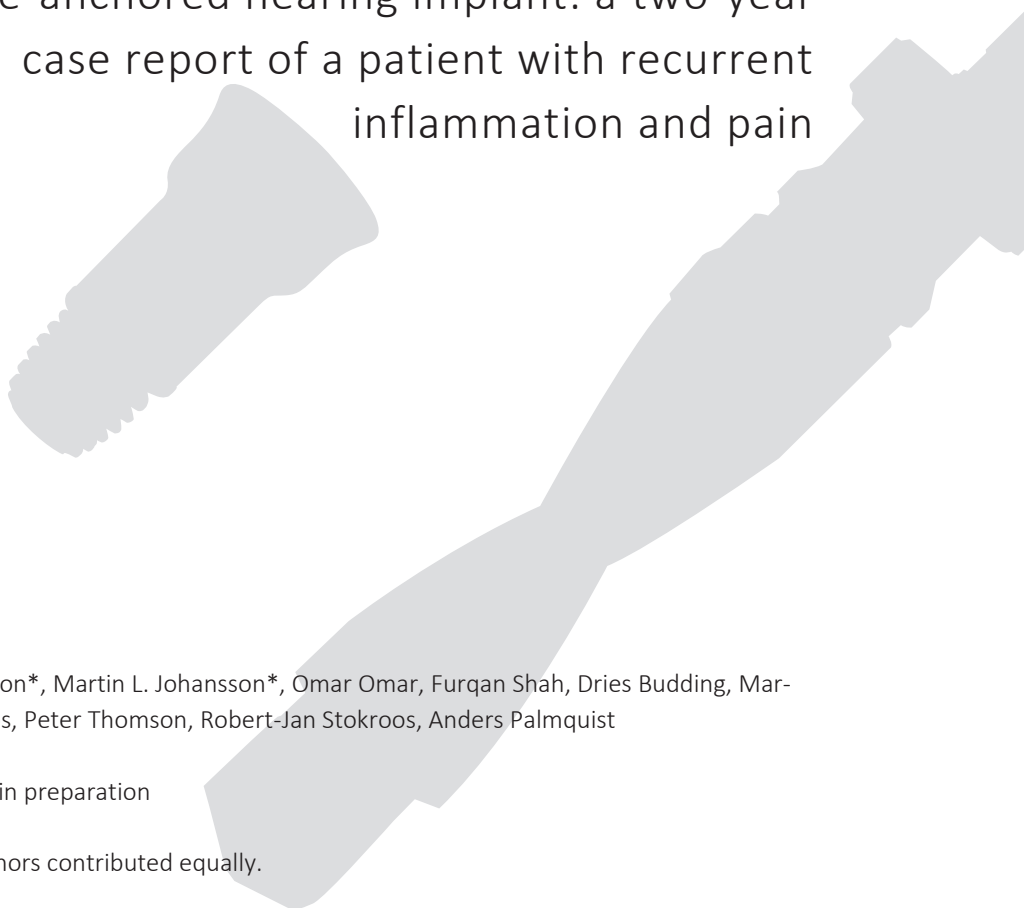
Chapter 8

Multimodal analysis of the tissue response to a bone-anchored hearing implant: a two-year case report of a patient with recurrent inflammation and pain



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Manuscript in preparation

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Abstract

Osseointegration is a well-established concept used in various applications in humans including the percutaneous Bone Anchored Hearing System (BAHS) for auricular rehabilitation. To date, few retrieved implants have been described. In this manuscript we present the case of a 39-year old patient who received a BAHS for mixed hearing loss. After initial surgery, several episodes of soft tissue inflammation were observed accompanied with pain leading to elective abutment removal 14 months post-surgery. Two years post-implantation, the BAHS implant was removed due to pain complaints. Histology showed clear evidence of osseointegration. These results were confirmed by micro-CT, Backscattered-SEM and Raman imaging. The molecular analysis by IS-pro™ showed polymicrobial colonization on the abutment over time, and on the implant, which included *Staphylococcus aureus* and *Staphylococcus epidermidis*. Fluorescence *in situ* hybridization (FISH) confirmed the localization of *S. aureus* and coagulase-negative staphylococci in the skin tissue. Histology showed evidence for bacterial colonization of the skin, and bacterial presence at the peri-implant bone is suggested.

This case report shows clear evidence of osseointegration from the analyses of the retrieved Ponto Wide Implant using several techniques. A large amount of bone was observed around most threads. We present a case suggesting that chronic pain related to the BAHS can result from a chronic bacterial infection with observed intra-cellular bacteria, even when macroscopically no signs of infection are present.

Keywords: BAHS, BAHA, Histology, IS-pro, osseointegration, chronic pain, infection, Holgers Index.

Introduction

Following the development of titanium implants for dental rehabilitation, in 1977 the bone-anchored hearing system (BAHS) was introduced as an option for audiological rehabilitation [1]. The BAHS consists of an osseointegrated implant placed in the temporal bone mounted with a percutaneous abutment. Over the last decades, the BAHS has become an established form of hearing rehabilitation for subjects suffering from conductive or mixed hearing loss and single sided deafness [2].

Overall, the BAHS is a successful treatment option with good clinical outcomes. Possible complications include, soft tissue inflammation and infection next to the implant, pain, loss of skin sensibility and implant loss. Implant removal is relatively rare. Therefore, to date few retrieved implants have been investigated [3–9]. This case report provides a detailed analysis of a retrieved BAHS. Here we aim to provide a detailed characterization of various compartment in BAHS and provide a unique evidence for pain complaints related to implantation. Various techniques including X-ray micro-computed tomography (micro-CT), histology, IS-pro™ (bacterial analysis), backscattered electron scanning electron microscopy (BSE-SEM), Raman spectroscopy, and fluorescence *in situ* hybridization (FISH) were employed.

Case presentation

We present a 39-year old Caucasian female who was treated for mixed hearing loss of the left ear by receiving a 4-mm Ponto Wide implant (Oticon Medical, Askim, Sweden) mounted with a 12-mm abutment in February 2015. Otological history included several ear surgeries leading to the creation of a radical cavity on her left side. Medical history included type-2 diabetes, treated with liraglutide (Victoza, Novo Nordisk, Denmark) and metformin since 2008 and 2010 respectively. High cholesterol has been treated with Crestor (AstraZeneca, United Kingdom).

After successful implantation, the implant stability quotient (ISQ) was determined to 51 using Ostell ISQ equipment (Ostell AB, Gothenburg, Sweden). The ISQ value rose during follow-up and remained stable at 57 up to a year following surgery (**Table 1**). The local, macroscopic status of the skin surrounding the percutaneous abutment was assessed using the Holgers Index [10]. During follow-up, the patient experienced adverse soft-tissue reactions with two episodes of Holgers score 2 (at 6 and 11 months) and one with Holgers score 3 (at 14 months) registered (**Table 1, Figure 1** a). In an attempt to reduce the adverse reactions, firstly treatment with topical antibiotic cream (NAME, COMPANY) was applied and finally the abutment was removed in May 2016, leaving the implant in the bone for a possible future reinstallation of an abutment. After removal of the abutment the skin healed, closing the wound over the implant (**Figure 1** b), however the patient experienced episodes of pain leading to elective implant removal surgery in March 2017 (**Figure 1** c, d).

Methods and Materials

Ethics

The patient was enrolled in a multicentre randomized controlled trial comparing the linear incision technique with soft tissue preservation to the Minimally Invasive Ponto Surgery (MIPS) technique for BAHS implantation [11]. The study was approved by the medical ethics committee (METC, azM/UM, Maastricht, The Netherlands) at Maastricht University Medical Centre+ (MUMC+) (NL50072.068.14). Additional consent was provided by the responsible ethics committee at MUMC+ for the retrieval of the BAHS implant. The patient provided additional written informed consent for this case study.

Sample collection and retrieval

Prior to implant surgery, at 12-week follow-up and during episodes of adverse soft-tissue reactions and inflammation (Holgers score ≥ 2), bacterial swabs were collected according to the previously mentioned study protocol. Soft tissue biopsies were collected at baseline, 12-week follow up and during each episode of inflammation (**Table 1**, **Figure 1** e, f).

At the time of implant removal, a 5-mm biopsy of the soft tissue above the submerged implant was retrieved and immersed in 4% paraformaldehyde solution, bacterial swabs were obtained from the cover screw and the top of the implant (**Figure 1** g, h). The implant and surrounding bone was retrieved *en bloc* using a trephine and immediately submerged in 4% paraformaldehyde solution. The soft tissue biopsy and the implant were transported to the Department of Biomaterials at the University of Gothenburg (Gothenburg, Sweden).

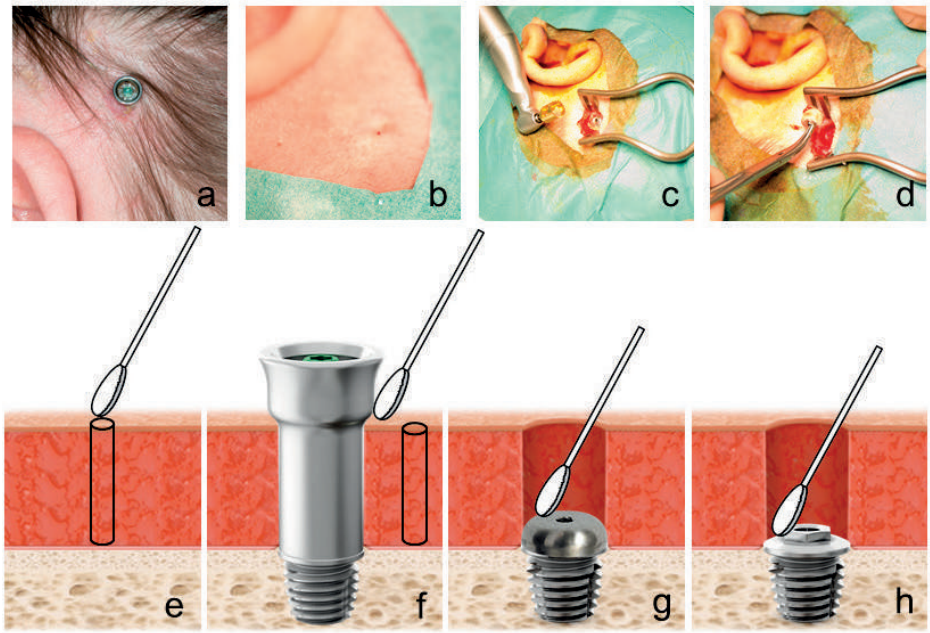

Figure 1. (a) The BAHS with mounted abutment at 12 weeks follow-up. In the lower left quadrant, a reddish moist irritated site can be observed. (b) The skin after abutment removal. No signs of inflammation are observed. (c) The implant in the bone during removal surgery. On the left: Trephine used to remove the implant is shown. (d) Retrieved implant with surrounding bone. (e) Illustration of sampling performed at baseline prior implantation where a skin swab (2x2 cm) is taken at the intended implantation site, prior retrieving a 1 mm soft tissue biopsy using a 1 mm biopsy punch, (f) at 12 weeks, and during episodes with adverse skin reactions a swab from the external side of the abutment and the peri-abutment skin are taken and a 1 mm skin biopsy is retrieved. Finally, at implant removal swabs are taken on the cover screw (g) and the top of the implant (h).

Table 1 Clinical outcome, sampling and treatments at the visits.

Visit number	Time[a)]	Reason for visit	Holgers[b)]	ISQ High	ISQ Low	Pain around implant[c)]	Radiating pain[c)]	Headache[c)]	Biopsy	Swab	Treatment, medication and comments
1	0d	Surgery	-	52	51	-	-	-	Yes	Yes	Terra-Cortril on ribbon for nine days with healing cap.
2	9d	Standard follow-up visit	0	55	53	1	0	0	No	No	Wound dehiscence, therefore Terra-Cortril ointment.
3	20d	Standard follow-up visit	0	53	53	0	0	0	No	No	Slight dehiscence.
4	24d	Pain after cleaning of abutment	0	53	52	6	0	1	No	No	Paracetamol if needed.
5	12w	Standard follow-up visit	1	-	-	0	0	0	Yes	Yes	Crust formation. Uses Terra-Cortril ointment 2 times per week.
6	6.3m	Pain around abutment	2	57	56	2	0	3	Yes	Yes	Terra-Cortril ointment (3 times per day for 1 week).
7	6.7m	Check after Holgers 2	1	56	56	0	0	0	No	Yes	Skin better. IS-PRO after Terra-Cortril treatment.
8	11m	Pain after wearing sound processor and skin irritation	2	58	57	1	0	5	No	Yes	Continuous inflammation under Terra-Cortril use. Revision surgery planned.
9	11.7m	Revision surgery under local anaesthesia	-	57	57	-	-	-	No	No	Terra-Cortril on ribbon with healing cap (1 week).
10	12m	Check-up after revision	0	57	57	0	0	2	No	No	Terra-Cortril ointment (1 time per day 1 week).
11	13.6m	Standard follow-up visit	1	57	57	1	0	2	No	No	-
12	14.3m	Irritation complaints	3	57	57	2	3	0	No	Yes	Removal of abutment with placement of cover screw.
13	15m	Check-up 2 weeks after abutment removal	-	-	-	0	0	0	No	No	Pain after abutment removal lasting 1.5 weeks.
14	25.5m	Implant removal surgery	-	-	-	-	-	-	No	Yes	Implant explantation

a) Days(d), weeks (w) or months (m) after surgery

b) Holgers Index: 0 No irritation; 1 Slight redness; 2 Red and slightly moist tissue, no granuloma formation; 3 Reddish and moist; sometimes granulation tissue; 4 Removal of skin-penetrating implant necessary due to infection [11].

c) Pain scores is graded in a 10-point scale. 0 representing “no pain” to 10 representing “the worst pain imaginable".

- Values not obtained or available

Sample preparation and analyses

The bacterial swabs were processed using a molecular technique, IS-pro (IS-Diagnostics, Amsterdam, The Netherlands) which uses the 16S-23S rRNA InterSpace region to classify Operational Taxonomic Units [12,13].

The retrieved samples fixed in paraformaldehyde, were dehydrated in a graded series of ethanol. The implant-bone sample was resin embedded in LR White (London Resin Co. Ltd, UK), while the skin sample was embedded in paraffin.

The intact plastic embedded specimen was scanned in a Skyscan 1172 (Bruker micro-CT, Kontich, Belgium) micro-CT system operating at 100 kV. The resolution was set to 5.88 µm, with 5 image averaging for each 0.4° rotation step. The projection images were reconstructed by back-projection, manually aligned along the long axis of the implant, evaluated in terms of bone growth in the threaded volume and visualized in the associated program suite (NRecon, Dataviewer, CTAn, CTVox and CTVol). In brief, a volume of interest was defined as a tapered cylinder encompassing the threaded area of the implant, wherein the bone volume was segmented by manual global thresholding based on the morphology [14], prior to performing a 3D analysis. The binary, segmented images of the implant, region of interest and bone within the region of interest were saved. A section matching the histological ground section was found by manual alignment of the saved segmented data-set and the 3D segmentation was directly compared to the histomorphometry by a 2D analysis of the matching section.

Following micro-CT scanning, 50 µm thick central ground sections were prepared by sawing and grinding (EXAKT® Apparatebau GmbH & Co, Norderstedt, Germany) and subsequently stained with toluidine blue or May-Grünewald Giemsa staining.
Qualitative histology and quantitative histomorphometry were performed to determine the amount of bone-implant contact (BIC) and bone area (BA) within the implant threads, using light optical microscopy (Nikon Eclipse E600; Nikon NIS-Elements software).

The remaining resin embedded bone-implant block was wet polished with 400–4000 grit SiC grinding paper. The samples were air-dried overnight prior to low-vacuum BSE-SEM imaging in a Quanta 200 environmental SEM (FEI Company, The Netherlands) operated at 20 kV and 0.5 Torr water vapour pressure.

Raman imaging was performed using a confocal Raman microscope (WITec alpha300 R, Ulm, Germany), equipped with a 532 nm laser, as described previously [15,16]. Briefly, the laser was focused down on to the sample surface using a ×10 objective having a numerical aperture of 0.25. Spectra were collected in the 300–1800 cm^{-1} spectral range using an electron multiplying charge coupled device (EMCCD) detector cooled to -60°C, behind a 600 mm^{-1} grating, at a spectral resolution of ~4 cm^{-1}, integration time of 2 s per pixel, and pixel size of 2 µm × 2 µm.

Fluorescence *in situ* hybridization (FISH) was performed in the soft tissue sections using a *S. aureus*-CoNS specific peptide nucleic acid (PNA) probe kit (KT005, AdvanDx A/S, Denmark), for the identification and localization of *S. aureus* (as green) and coagulase-

negative staphylococci (CoNS) (as red) directly in the tissue. A drop of PNA probe in hybridization solution was added to each tissue section, a cover slip was added, and slides were placed in an hybridization oven at 55°C for 90 min. Slides were subsequently washed in wash solution at 55°C for 30 min and air dried. Mounting medium and a cover slip was applied, and the stained slides were visualized in a fluorescence microscope (Eclipse E600, Nikon, Japan) and a confocal microscope (C2plus, Nikon, Japan) with a plan-apochromat 60x/1.2 water immersion objective. The excitation/emission spectra used were: blue filter (340-380/435-485 nm), green filter (465-495/515-555 nm), and red filter (540-580/600-660 nm).

The methods for q-PCR have previously been described [17]. Recently, results of q-PCR for subjects included at MUMC+ have been published [17]. In short: cDNA was obtained by isolation of RNA using TRI Reagent (Sigma, St. Louis, MO, USA). Approximately 750ng of RNA was used to transcribe to cDNA using the SensiFast cDNA Sythesis Kit. Expression for genes related to inflammation (IL-1ß, IL-6, TNF-α, TGF-ß, MIP-1α), tissue metabolism (TIMP-1, COL1α1), vascularization (VEGF, FGF-2), and bacterial infection (TLR-2) (Sigma-Aldrich, St. Louis, Missouri, USA) are presented. Relative expression was determined using LinRegPCR (version 2016.1).

Results

Micro-CT and histomorphometry

The micro-CT revealed large amounts of bone around the implant, mainly of cortical type with only a smaller amount of porosity in the trephined volume (**Figure 2** a). Some fractures originating from the retrieval are visible both close to the implant top as well as closer to the dura side. Lack of bone tissue was observed close to the implant in 6 out of 13 threads, especially in the thread valley region (**Figure 2** b). The 3D quantification of tissue in growth in the threaded volume of the implant showed a bone volume fraction of 86.4%. The segmentation was further compared to the histological measurement showing similar values for the 2D sections. Values were 80.8% for the micro-CT and 81.6% for histomorphometry, validating the 3D results (**Figure 2** b, c). The quantitative histomorphometry revealed a direct bone implant contact (BIC) of 57.7% and bone area within the threads (BA) of 81.6% (**Figure 1** c).

Histological evaluation of bone-implant sample

The qualitative histological assessment of the toluidine blue-stained sections showed the implant was well-integrated in dense, mature, recipient bone (**Figure 2** d, e). In general, bone appeared filling all the threads of the implant, and at many occasions in direct contact with the titanium surface at the light microscopy level. In some threads, soft tissue separated the implant surface from the surrounding bone in the interface.

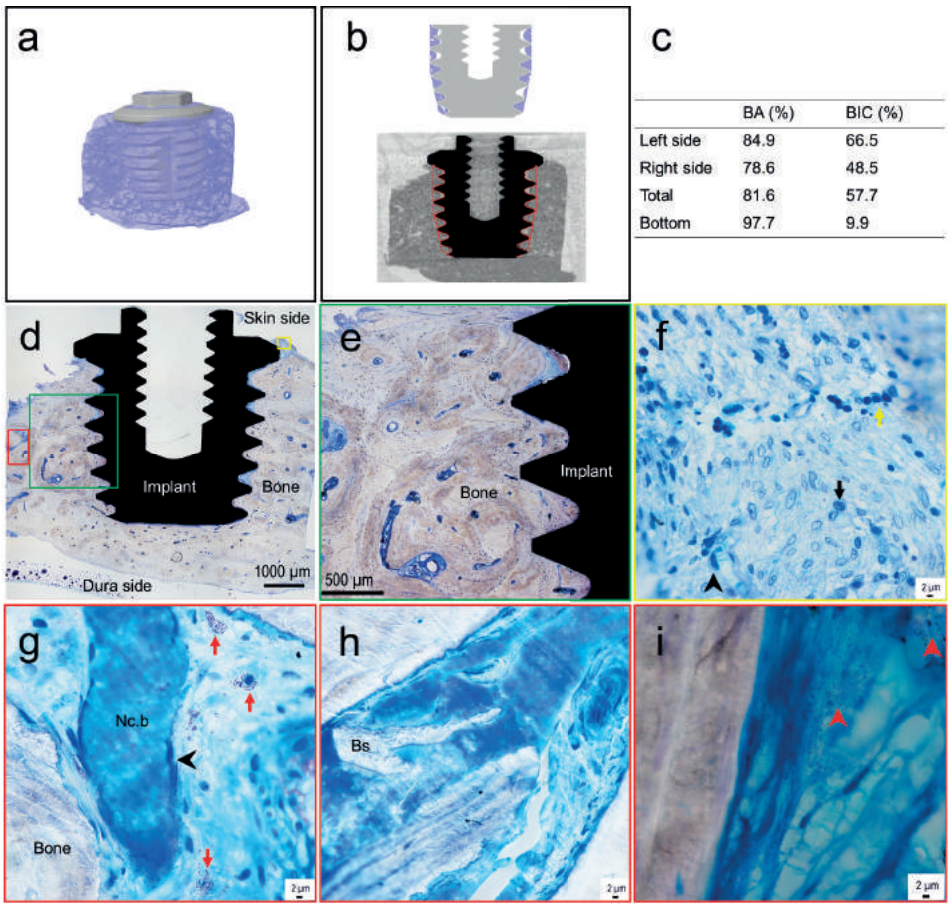

Figure 2 Micro-CT images, histomorphometry and histological assessment of the bone-implant sample of BAHS implant. (a) microCT 3D reconstruction of retrieved implant with surrounding bone (b) Region of interest and segmentation of the bone surrounding the implant (c) Histomorphometric data, BA=Bone area, BIC=Bone to implant contact (d) the toluidine blue stained-section shows the implant integrated in the recipient temporal bone site, with dense mature bone filling the implant threads and in contact with titanium surface in most of the threads. The green, yellow and red boxes in (d) represent selected regions presented at higher magnifications in (e), (f) and (g, h, i), respectively. (f) The toluidine blue stained-section at high magnification show an inflammatory infiltrate in the top region at the interface of bone with the top flange of the implant. The inflammatory infiltrate consists of mainly chronic inflammatory cells, containing mononuclear/macrophage, lymphocyte and plasma cell types (exemplified by the black arrowhead, black arrow and yellow arrow in (f), respectively. (g and h) At the several occasions, necrotic bone (Nc.b), and bone sequesters (Bs) were found in the cavities in the surrounding bone. The necrotic bone islets appeared always surrounded by spindle shaped elongated macrophages (exemplified by the black arrowhead in (g). Mast cells are frequently detected in the bone cavities (some are indicated by the red arrow in (g)). (i) The Giemsa-stained section shows the presence of bacteria-like microstructures (exemplified by the red arrowheads) in some cavities in the surrounding bone.

At high magnification (×600 water immersion objective), the top region of bone close to the implant flange revealed a considerable amount of inflammatory infiltrate, containing

mainly chronic inflammatory cells, including monocytic/macrophage cells, lymphocytes and plasma cells (**Figure 2** f). Whereas no polymorphonuclear cells (PMNs) were observed, mast cells and degranulating mast cells were found on several occasions in the cavities in the surrounding bone (**Figure 2** g). Further, in some occasions, small areas of necrotic bone spicules, surrounded by macrophages, (**Figure 2** g) and bone sequestra (**Figure 2** h) were detected within the surrounding bone, at distance from the implant. Moreover, the evaluation of the Giemsa-stained sections showed the presence of darkly-stained bacteria-like microstructures, sporadically found in some of the bone cavities at distance from the implant (**Figure 2** i).

BSE-SEM corroborated the histological observations. Within the implant threads as well as around the implant, large amounts of remodelled, highly mineralized, lamellar/osteonal bone was observed (**Figure 3**). Within the implant thread, Raman imaging revealed typical components of mature bone, i.e., apatite (v_1 PO_4^{3-} peak at 960 cm^{-1}, v_2 PO_4^{3-} band centered around 432 cm^{-1}, v_4 PO_4^{3-} band centered around 580 cm^{-1}, and v_1 CO_3^{2-} peak at 1070 cm^{-1}) and type-I collagen (amide III band at 1215–1300 cm^{-1}).

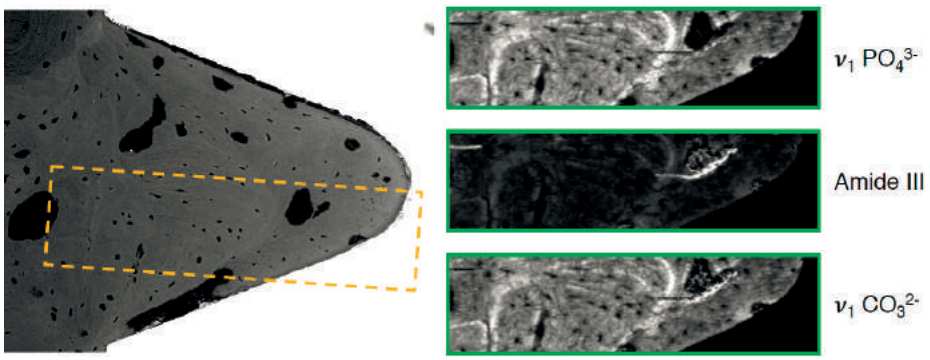

Figure 3 Backscattered electron scanning electron microscopy (BSE-SEM) and Raman imaging corresponding to the yellow box, showing the distribution of phosphate (v_1 PO_4^{3-}), collagen (Amide III), and carbonate (v_1 CO_3^{2-}) within the implant thread.

Histological evaluation of the soft tissue sample

The histological assessment of the skin sample, which had grown over the BAHS implant showed a normal appearance of the epithelial layers of the epidermis and the subepithelial, vascularized, connective tissue dermis (**Figure 4** a). Clusters of darkly stained bacterial aggregates were frequently detected in the dermal connective tissue (**Figure 4** a, b and c). Variable degrees of inflammatory infiltrates were detected, consisting predominantly of mononuclear/macrophage and lymphocyte cell types, and to a lesser degree plasma and mast cells. However, PMNs were seldom detected (**Figure 4** b, c, e, f). Some of the inflammatory cells assumed interaction with the bacterial cells, indicated by the close proximity to the bacterial aggregates as well as presumed intra-cellular bacteria (**Figure 4** b). In some occasions, relatively dense bacterial aggregates were also found within the

skin appendages, in association with the hair follicles (**Figure 4** d). FISH analysis of soft tissue further confirmed the presence of bacteria, as single cells and clusters, across the epidermis and dermis (**Figure 4** g, h and i). Both *S. aureus* (green) and CoNS (red) were identified in the soft tissue, as observed by the strong green and red fluorescent cocci (≤1 µm) over the autofluorescent soft tissue in the background.

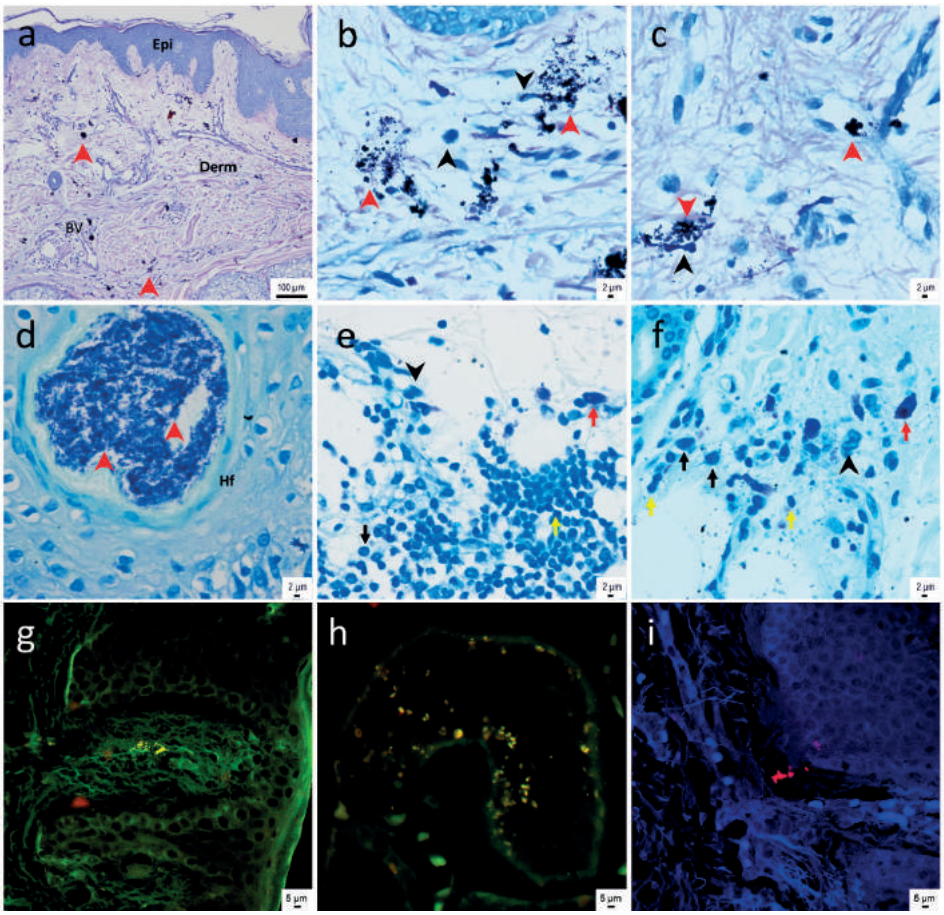

Figure 4 Histological assessment of the skin sample above the BAHS implant. (a, b, c) Giemsa-stained histological sections show normal appearance of skin structure, consisting of epidermis (Epi) and subepithelial connective tissue dermis (Derm) containing blood vessels (BV). Aggregates of bacteria (indicated by the red arrowheads in a, b, c) as well as mononuclear/macrophage cells (indicated by the black arrowheads in b, d) are frequently detected in the connective tissue. Some of the bacterial cells assume intra-cellular localization (b). The toluidine blue-stained sections also show dense bacterial aggregates in association with hair follicles (red arrowheads in d). (e and f) Relatively dense inflammatory infiltrates are found in deepest part of the soft tissue sample, presumably interfacing with the bone where the implant is inserted. The inflammatory infiltrate in (e and f) appears to contain mononuclear cells/macrophages, lymphocytes, plasma cells and mast cells (indicated by black arrowheads and the yellow, black and red arrows, respectively). (g-i) Tissue sections underwent fluorescence in situ hybridization (FISH) with a peptide nucleic acid (PNA) probe targeting coagulase-negative staphylococci

(CoNS) and Staphylococcus aureus in the tissue. (g, h, i) CoNS (red cocci of approximately 1 µm) were detected in the tissue, both superficially at the epidermis (g) and at deeper layers of the dermis (h, i). (g, h) S. aureus (green cocci of approximately 1 µm) was also detected in the tissue.

Microbiological identification by IS-pro™

IS-pro™ analyses are presented in **Figure 5**. The analyses showed that at baseline mainly skin bacteria were present such as *Propionibacterium acnes*, *Staphylococcus capitis*, *Staphylococcus epidermidis*, and *Streptococcus pneumoniae/mitis*. At 12-weeks follow-up only *Staphylococcus epidermidis*, *Staphylococcus hominis* and *Streptococcus pneumoniae/mitis* were observed. In the following months two episodes of inflammation were observed. During these episodes of inflammation, more bacterial species were observed including *Haemophilus parainfluenza*, *E. coli* and *Enterococcus faecalis* (**Figure 5**). During visit 12, the abutment was removed. Compared to previous visits, a polymicrobial flora was observed including high amounts of *Staphylococcus aureus* and *Finegoldia magna* (**Figure 5**). During visit 14, a similar polymicrobial flora was found on both the cover screw and implant with high quantities of *Staphylococcus aureus.*

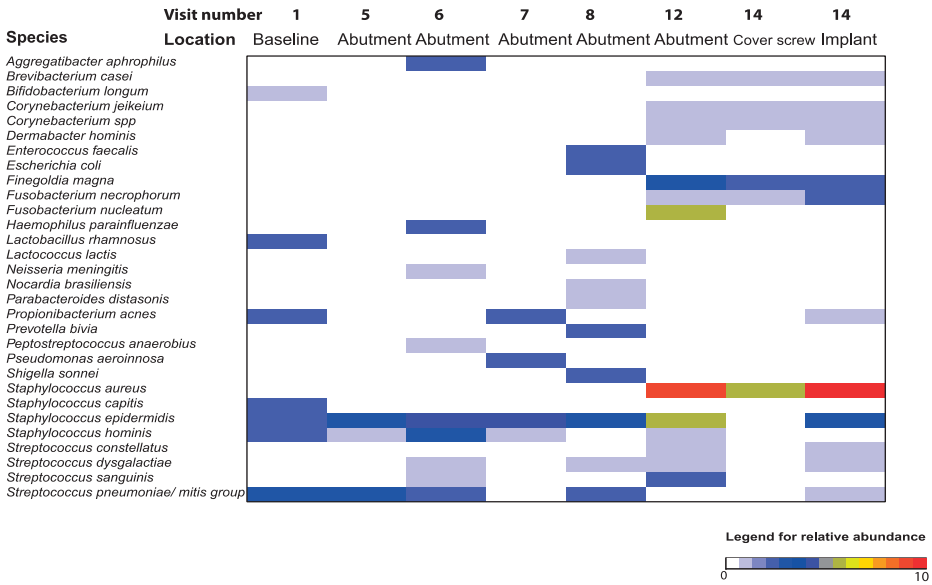

Figure 5 Relative expression of bacteria on BAHS over time. Colour indicates relative abundance of specific species at each timepoint with red indicating the highest expression. Location indicates the area where a cotton swab was taken. At baseline, a swab of the intended implantation site was obtained. Abutment indicates a swab of the skin penetrating abutment and approximately, 1 cm of peri-abutment skin around the abutment. Species indicates the respective bacterial species.

Molecular profile

q-PCR results are presented in **Figure 6**. During inflammation, IL-1β, IL-6, TNF-α, MIP-1α, FGF-2 and TLR-2 expression were all strongly increased compared to 12-weeks expression. TGF-β expression only moderately increased during inflammation. In contrast, TIMP-1, COL1α1 and VEGF expression decreased during inflammation compared to 12 weeks expression.

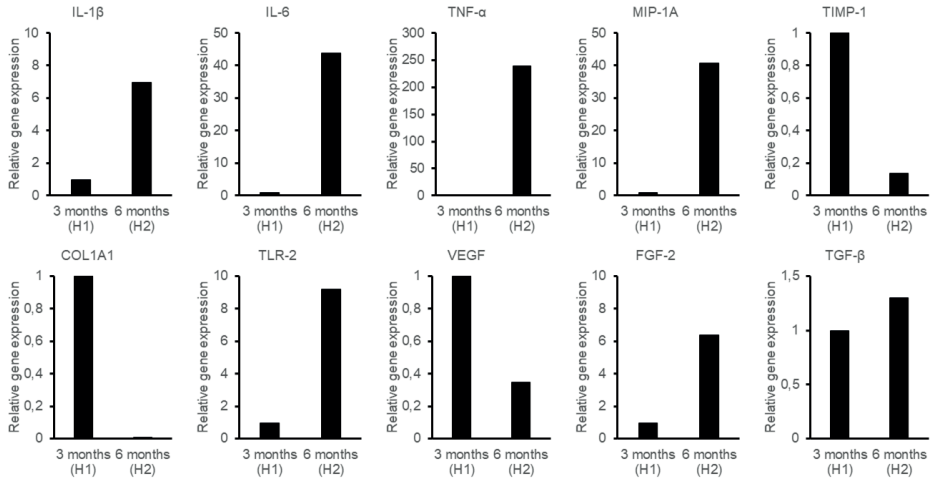

Figure 6 Qualitative PCR of cytokine expression. H1 indicates Holgers 1 scoring according to the Holgers Index scoring. H2 indicates Holgers 2 scoring which was defined as an episode of soft tissue inflammation.

Discussion

During the last decades, the BAHS has become an established method of audiological rehabilitation with high implant survival rates [18–20]. Osseointegration is an established concept in BAHS, dental rehabilitation and femoral prothesis. However, to date few retrieved BAHS implants have been described [4–9]. Here we present an elaborate case report of a subject reporting chronic pain that persisted after abutment removal with a stable implant. This is the first case report describing chronic infection as a likely explanation for chronic pain.

Osseointegration

Histology, BSE-SEM and micro-CT all provided evidence that there was a high degree of osseointegration. Previous studies showed an estimated Bone-to-metal contact of 28%-88% for stable implants with increased values over time [4–7,9]. This is in line with the observed 57.7% BIC in this study. Under the flange, an area with less bone contact has been observed in most case reports [4,5,7]. The degree of bone to metal contact has not been systematically described in these cases. Visual assessment indicates that the retrieved implant presented here has a higher degree of bone to metal contact under the flange. Moreover, to date no retrieved Ponto Wide Implant has been investigated. Possibly, the higher degree of bone under the flange may be attributable to an improved implant design. However, this should be evaluated systematically in future studies.

Bacterial colonization

At baseline and at 12-week follow-up mainly normal skin bacteria were observed. However, during episodes of inflammation the bacterial diversity increased on the peri-abutment skin including other bacteria such as *E. coli*, *Haemophilus parainfluenzae* and *Enterococcus faecalis*. Only later on, during abutment removal, *Staphylococcus aureus* was observed in high amounts.

Strikingly, the bacterial profile on the implant strongly resembled the profile found on the abutment prior to removal, indicating that the infection was maintained on the implant level over 10 months of soft tissue healing. In addition, the soft tissue contained several areas of persistent *S. aureus* bacteria as confirmed by FISH. Additionally, bacteria-like structures were observed in the peri-implant bone suggesting bacterial colonization in the bone.

The subject complained of episodes of pain with tenderness of the soft tissue around the implant, while macroscopically the skin and bone showed no clear signs of infection (**Figure1** b-c). The presence of bacteria and subclinical infection are plausible explanations for these complaints. Although, confirmation of these results is warranted (i.e. by culturing the causative pathogen).

Clinical considerations

Chronic pain related to BAHS is a known problem that can hinder overall satisfaction, increases morbidity and medical consultation and can even lead to implant removal [20–22]. Titanium allergy, dura contact and bacterial presence have been postulated as a possible explanations for these complaints [5,7,23]. Kruyt *et al.* suggest to treat patients with oral antibiotics in case of chronic pain [24]. This recommendation is supported by our findings.

During follow-up, pain complaints were present although the bacterial species altered over time. Previously, in dental implants pain was associated with IL-6 and IL-8 expression[25]. Although, we could not confirm these results for BAHS[17], a similar mechanism

might be attributable for pain complaints in this patient, irrespective of which specific bacterial species.

Bacteria were found in the peri-abutment soft tissue with indications of their presence also in the peri-implant bone. If we assume that a bacterial colonization is indeed present, it is striking that the implant itself was well integrated. Also ISQ, a suggested surrogate for implant stability, [26] remained stable over time. Immune responses around implants are assumed to be impaired [27,28]. Possibly, an equilibrium might be present preventing complete bacterial colonization of the implant whilst (intra-cellular) bacteria persist. Shifts in this equilibrium might explain the episodes of tenderness described by the patient.

Future studies

To gain further understanding of osseointegration, a detailed evaluation of clinically retrieved implants are needed. Elective implant removal is quite rare. However, when removal is requested it is usually associated with a relative high degree of morbidity. Although uncommon, chronic pain is one of the most common reasons for implant removal and may benefit from increasing the knowledge on the causes. Biofilms have been shown to be present on BAHS [3,29]. Implant-associated infections can pose a challenge in clinical practice, especially in cases of biofilm formation leading to chronic infections and increased resistance to antibiotic treatment [30]. Device removal may be necessary in these cases for the complete eradication of the infection. As presented in this article, several techniques can be employed in cases of implant removal to increase knowledge on osseointegration, chronic pain and implant-associated infection. Larger case series using various techniques to determine osseointegration and sensitive procedures for bacterial detection are needed to confirm our results.

Diagnostics

A dilemma presents itself considering the best method for diagnosis of chronic (peri-)implant infection without the removal of the device itself. The BAHS is a percutaneous device resulting in a permanent bacterial colonization through the skin breach. Both conventional bacterial cultures and IS-pro™ seem suitable techniques for the detection of bacteria on BAHS [31]. Knowledge on which bacterial species are present at the implant level in patients without pain is lacking. Due to the titanium composition of the BAHS, imaging by means of MRI or CT is unlikely to yield reliable outcomes with respect to chronic bone inflammation. Possibly, histological evaluation of soft tissue biopsies taken from the peri-abutment might yield some evidence of soft tissue invasion with bacteria and inflammatory cells. Recently, the use of paperpoints, a less invasive sampling technique, has been shown feasible to evaluate peri-abutment bacteria[31]. The composition of inflammatory cells and cytokine expression profiles could provide more insight in the diagnosis of infection or inflammation of the peri-abutment skin. Although, one should

consider the risks for complications of a biopsy, multimodal evaluation of soft tissue combined with a peri-implant bone biopsy could yield the most conclusive evidence for chronic infection of the peri-implant bone.

Conclusion

This case report shows clear evidence of osseointegration using several techniques on a retrieved Ponto Wide Implant. A large amount of bone was observed around most of the threads. We present a case suggesting that chronic pain related to the BAHS can result from a chronic bacterial infection with observed intra-cellular bacteria, even when macroscopically no signs of infection are present.

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Part III

Summary and discussion

Summary and discussion

The Bone Anchored Hearing System (BAHS) serves as a treatment option for the rehabilitation of several types of hearing loss[1]. Diverse techniques for BAHS implantation -- such as the linear incision technique with implant placement in the incision[2], the linear incision technique with soft tissue preservation[2–4] and the Minimally Invasive Ponto Surgery (MIPS) technique -- have been used in recent years [4,5]. Currently, there is a trend towards use of soft tissue preservation techniques[2]. This thesis describes various clinical and more explorative endpoints regarding BAHS surgery, BAHS outcomes and soft tissue complications.

Part I:

Chapter 2 describes the design of a multicenter randomized controlled trial comparing the linear incision technique with soft tissue preservation [3,6] to the MIPS technique [5–7] . The aim of the study was to investigate clinical outcomes such as inflammation, infection, loss of skin sensibility, pain, skin dehiscence, extrusion rate, stability values, processor use and complications. After defining the outcomes, the chapter presents a set-up for a cost-utility analysis and explorative measures to extend the understanding of tissue reactions related to the BAHS. Time is specified as surgical procedural time and time spent in the surgical theater. Pain scores for direct pain near the BAHS were obtained using an analog scale (0-10). Radiating pain and headache associated with the BAHS were noted as well. Loss in skin sensibility next to the implant was quantified in millimeters. Skin sagging was investigated systematically per quadrant, as was skin height. Patients provided a self-reported assessment of cosmetic effect, both with and without the processor attached (**chapter 3**). Overall, this study included several new or modified outcome measures to improve the comprehension of BAHS-related outcomes.

Chapter 3 reports the short-term (12-week) outcomes of the study that was described in **chapter 2**. At 12 weeks, the groups showed no difference in rate of peri-abutment dermatitis/inflammation (9.1% MIPS vs. 16.7% linear incision, p=0.37) for the primary outcome variable. Several outcomes were better for the MIPS group compared to the linear incision group. Surgery time, skin sensibility, skin sagging and observer-rated cosmetic effect were all favorable for the MIPS group. No significant differences were found for implant loss or for pain scores, including pain around the implant, radiating pain and headache. During the 12-week follow-up period, more implants were extruded in the MIPS group than in the linear incision group (n=4 (12.1%) vs. n=1 (3.3%)). This observed difference warrants further study. A complete overview is given of events and complications encountered during follow-up. One observation was a new event that, to our knowledge, had not been described before: trouble sleeping on the implant side of the head. Future studies should be conducted to investigate the severity, frequency and duration of this event. ISQ was significantly influenced by surgical treatment, abutment

length and surgical technique. ISQ values were 2.3-2.8 points lower in the MIPS group than in the linear incision group with soft tissue preservation. This divergence might be explained by differences in osteotomy between surgical techniques. No clear relationship could be identified between lower ISQ values at surgery and future implant loss during follow-up.

Chapter 4 presents the implant survival rates for 550 primary BAHS placements that were performed at Maastricht University Medical Center (MUMC+) from 1991 to January 2017. Overall, 6.2% of the implants were lost. Long-term survival rates were as high as 92% after 15 years of follow-up when using 4-mm implants. Male gender (HR=1.99, 95%CI=1.00-3.95, p<0.05), age < 18 years (HR=3.43, 95%CI=1.38-.8.52, p=0.008) and second 4-mm implant (HR=5.67, 95%CI=1.94-16.54, p<0.002) were associated with a higher risk of implant loss. In contrast, 3-mm implants (HR=1.68, 95%CI=0.61-4.67, p=0.32) and age > 60 years (HR=0.59, 95%CI=0.31-1.61, p=0.41) were not associated with implant loss. Spontaneous loss and trauma were frequently reported as reasons for implant loss. In two cases, implants were electively removed. The relatively low number of implant losses hindered an evaluation of the effect of a learning curve for surgeons and differences between surgeons. Young age, advanced age and smoking have previously been described as risk factors for implant loss in BAHS [8–10]. A meta-analysis by Chen *et al.* revealed that smoking and irradiation are associated with increased risk for implant loss in dental implants, whereas the relationship between diabetes and osteoporosis is less clear [11]. The risk of implant loss in relation to implant width [12,13], design[14] and early loading [15,16] warrants further study.

Part II:

In MIPS and other skin preservation techniques the visibility of the implant-bone interface can be obstructed. Flapless surgery for dental implants has been associated with increased implant loss rates[17]. Bone overheating and partial seating were postulated as possible issues[17]. Consonant with dental implants, the same issues might apply to MIPS [4]. In recent years, Cone Beam CT (CBCT) has been performed to evaluate cochlear implant position, and the procedure is routinely performed before placing dental implants[18,19]. **Chapter 5** presents an evaluation of the use of CBCT imaging for BAHS implant seating *in vitro* and *in vivo.* CBCT imaging seems to allow for qualitative visual inspection of the bone-implant interface *in vivo* and *in vitro*. The cut-off value between fully and partially seated BAHS was estimated at 0.49 mm for the distance from CBCT rim to bone surface. In one subject, it was possible to visually identify an angulated seating, which was extruded during follow-up. This case does not necessarily reflect a causative relationship between angulated/partial insertion and implant loss. However, it does provide evidence that CBCT could be of value in BAHS research. Moreover, *in vitro* it was found that the implant stability quotient is influenced by insertion depth ($p<0.001$), abutment length ($p<0.001$) and artificial bone density ($p=0.024$).

The influence of BAHS implantation and peri-abutment skin reactions on cytokine expression is presented in **chapter 6**. Cytokines related to inflammation, tissue remodeling, microbial infection and vascularization were selected [6]. qRT-PCR was performed to determine mRNA expression of IL-1β, IL-6, IL-8, TNFα, IL-17, IL-10, TGF-ß, MIP-1α, MMP-9, TIMP-1, COL1α1, VEGF-A, FGF-2, TLR-2 and TLR-4. The analyses showed that mRNA expression was significantly up-regulated for IL-1β, IL-8, MMP-9, TIMP-1 and COL1α1. Expression of IL-6 and FGF-2 was significantly down-regulated after implantation. Within subjects, no difference was observed in expression during inflammation compared with expression 12 weeks post-implantation. Between patients, expression of IL-1β and IL-17 was significantly higher during inflammation compared to subjects without inflammation at 12-week follow-up. TNF-α and TLR-2 showed a trend towards up-regulation during inflammation. Holgers 1 scorings showed a strong negative correlation compared to Holgers 0 scorings for TNF-α. Smoking was associated with increased expression of MMP-9. This study showed that markers related to inflammation and tissue remodeling were up-regulated at 12 weeks post-surgery compared to the pre-surgical situation. BAHS implantation might result in a continuous activation of several cytokines.

The skin microbiome within 44 subjects is prospectively evaluated in **chapter 7** using IS-pro™, a 16S-23S molecular sequencing technique[20]. Swabs were obtained at baseline, 12 weeks post-implantation and during episodes of inflammation. Sample similarities within subjects were found to be stronger than between subjects at baseline before surgery. The similarities between abutment microbiome at 12 weeks and pre-surgery microbiome were higher within subjects than between subjects. The Shannon Diversity Index was found to have increased on the abutment swabs 12 weeks post-implantation

compared to implantation swabs for Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia (FAFV), but not for all bacteria, Bacteroidetes and Proteobacteria. *FAFV* includes skin bacteria. No differences were observed for correlations or the diversity index in non-inflamed abutments compared to inflamed abutments. No differences in the baseline diversity index could be observed for subjects prone to inflammation. Several bacteria were observed on the normal skin at baseline and post-implantation. *Staphylococcus epidermidis, Streptococcus pneumoniae/mitis* and *Haemophilus parainflenzae* were mainly observed on the post-surgical abutments. Compared to the pre-surgical baseline swabs, *Propionibacterium acnes* and *Staphylococcus capitis* were observed less often on swabs 12 weeks post-implantation. A possible explanatory factor is the use of Terra-Cortril containing hydrocortisone, oxytetracycline and polymyxin-B, as it may have led to the relative overgrowth of *Staphylococcus epidermidis*. During inflammation, *Staphylococcus aureus* was observed more often, although there was no statistically significant difference. Moreover, the relative presence of *Staphylococcus aureus* had strongly increased. When prescribing antibiotic ointments, it may be fruitful to prescribe antibiotics that are effective against these bacteria.

Currently, knowledge of the cellular and molecular mechanisms that lead to adverse events such as peri-implant inflammation or chronic pain after BAHS implantation is limited. **Chapter 8** presents a case of explantation after two years of follow-up. The BAHS was explanted due to chronic pain without macroscopical signs of inflammation. For this subject, cytokine expression, microbiological data, histology, micro-CT and clinical data was available. Osseointegration of the fixture in the temporal bone was demonstrated using light microscopy and ultrastructural techniques. The microbiological analysis showed the presence of *Finegoldia magna*, *Fusobacterium nucleatum*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus sanguinis* on the abutment. The same bacteria were found on the cover screw and implant, while the skin had healed nicely over a period of several months. Of these bacteria, *Staphylococcus aureus and Staphylococcus epidermidis* were the most abundant species. Even though, macroscopically, no evidence of inflammation was visible Staphylococcus aureus and Staphylococcus epidermidis were also detected in the skin that healed over the implant. Moreover, there were suggestions of bacteria in the peri-implant bone. This report indicates that an ongoing chronic infection of the implant could be a cause for chronic pain.

General Discussion

The present thesis is mainly built on one of the few available multicenter randomized controlled trials of BAHS. For the first time, this study provides a direct comparison of the surgical techniques used in clinical practice. Successive chapters contain the first prospective studies to investigate the influence of BAHS implantation on cytokine expression and the skin microbiome using molecular techniques. One study concerned the use of CBCT as a research technique for BAHS seating. Another study gave an overview of implant loss rates at the study site, MUMC+. The retrieved implant described in **chapter 8** provided the first evidence for chronic infection that could be associated with chronic pain complaints, a rare and poorly understood complication related to BAHS [21,22]. Several limitations of the research material underlying this thesis should be mentioned. Ideally, the randomized controlled trial would have been blinded. Due to practical considerations, however, this was not the case. Additionally, the sample for the subjects described in **chapter 3, chapter 5 and chapter 6** was limited in size. A larger sample of implant losses as described in **chapter 4** would have allowed for more extensive analyses. In future studies, blinded randomized controlled trials could be attempted when comparing implants, abutments and possibly even surgical techniques. To improve the power of BAHS studies, sample sizes should be increased to a level at which they become adequate for detecting small to moderate effects. But because any one center performs relatively few BAHS surgeries per year, an alternative approach might include the use of new designs such as adaptive clinical trials. The Bayesian statistics employed with such designs might allow BAHS trials to be performed with limited sample sizes [23].

Outcome measures

The Holgers Index was introduced in 1988, based on clinical observations. Currently, the Holgers index is the outcome measure related to soft tissue status in BAHS. In **chapter 6**, it was pointed out that the difference between Holgers 0 and Holgers 1 scorings are negatively correlated to TNF-α. Kruyt *et al*. recently created the IPS, a new BAHS assessment scale to rate soft tissue reactions[24]. Unfortunately, no information is available regarding its biological validity. For future studies, additional information regarding inter- and intra-rater reliability of both the Holgers Index and IPS would be beneficial.

Chapter 2 mentions the lack of formal outcome measures for BAHS [6]. The interpretation of measures such as surgical duration and implant extrusion is quite straightforward. However, most other outcome measures have never been validated for BAHS. For example, there is no straightforward definition of or consensus on s*kin sagging*. Patient-centered outcome measures are of increasing interest in general medicine. Nonetheless, most measures in BAHS are clinician-centered.

In 1990 Buser *et al.* gave five criteria by which treatment of dental implants could be considered successful: "1: Absence of persistent subjective complaints such as pain,

foreign body sensation and/or dysesthesia; 2: Absence of a recurrent peri-implant infection with suppuration; 3: Absence of mobility; 4: Absence of a continuous radiolucency around the implant; 5: Possibility for restoration"[25] . Outcome measures such as probing depth, bleeding, swelling, plaque index, minor complaints, major complaints, esthetics, function, discomfort, patient satisfaction with appearance and general satisfaction were mentioned as well. Levi *et al.* postulated that patient satisfaction with overall treatment should be good or excellent before a dental implant can be considered successful [26]. The scar assessment scale, POSAS has been used as a measure for BAHS satisfaction concerning esthetics [2,27,28]. However, a measure of patient satisfaction with the overall treatment is lacking for BAHS. Consensus on what constitutes successful treatment in BAHS would be beneficial to the research field. In 2014 the auditory rehabilitation network was established with the objective of preparing a standardized validated outcome set for BAHS endpoints [29]. That network could serve as a platform for defining selected outcome measurements and formulating a single definition for treatment success in BAHS, taking the perspectives of the clinician as well as the patient into account.

Minimally Invasive Ponto Surgery

MIPS was designed to standardize surgery, minimize soft tissue damage and improve outcomes related to BAHS [30]. In 2016 Johansson *et al.* presented the results of the first 76 patients that received a BAHS using MIPS [5]. Compared to soft tissue reduction and skin-graft techniques, results have improved considerably. A complication such as flap necrosis has been eliminated. Moreover, numbness is rare after MIPS. For both the linear incision technique with soft tissue preservation and MIPS median pain scores are very minor or absent post-surgery. For MIPS, surgical timings have decreased even further. The surgical technique and short surgical duration make it suitable for an outpatient clinic procedure under local anesthesia. The classical techniques resulted in quite large esthetic defects that were permanent. After MIPS, the abutment can be removed resulting in a minor full thickness scar which results in better esthetics. Moreover, the full thickness scar eliminates the need for scar resection in case of implantation of a subcutaneous device in a later timepoint.

The report for the first 76 patients that received MIPS described three implant losses within three months following implantation. This is high compared to our clinical experience reported in **chapter 4**. In **chapter 3**, several implant losses were described using MIPS. During the trial described in **chapter 3**, the MIPS surgical kit was updated [7]. However, implant losses remain a concern [4]. Incomplete insertion, implantation in a suture line, soft tissue entrapment between the implant and bone surface, and inadequate cooling have all been proposed as potential risk factors [17,31,32]. Inadequate cooling and idle drilling in MIPS was shown to lead to higher temperatures in the area adjacent to the drilling hole [32]. Furthermore, the newly designed drills are much more efficient and provide more tactile feedback regarding the correct drilling hole. The learning curve for

surgeons to familiarize themselves with these new drills could play a role as well. These issues can come into play when a new surgical technique is being developed. Comparable experiences have been reported with the introduction of flapless surgery in dental implants [17]. One should be cautious about using new surgical techniques before they have been fully evaluated. MIPS is a promising technique that requires further development. Most importantly, we should all aim for open communication on issues that will inevitably arise with new surgical techniques.

Implant Stability

As reviewed by Nelissen *et al.,* ISQ has been an important outcome measurement in several BAHS investigations [33]. In this review, it was advised to follow a trend for a single patient. Currently, there are several implants available with various abutment lengths limiting the interpretation of a single solitary ISQ measurement without previous measurements. *in vitro* bone density seems to influence ISQ [34]. *in vivo* it is less straightforward to correlate ISQ values to bone quality or, more importantly, to clinical outcomes such as implant loss [14,35]. The complex interplay between surgical technique, insertion torque, follow-up timing, abutment length, bone quality and osseointegration has thus far limited our understanding of ISQ [36]. The patients mentioned in **chapter 3** who suffered from implant loss during follow-up had relatively high ISQ values during surgery. A decline was seen in some but not all subjects before their implant was lost. Moreover, we do not know whether implant losses can be prevented by temporarily not loading the sound processor or by treatment with systemic antibiotics. Recently, a new implant stability testing device the ASIST was introduced with promising results [37]. The clinical value of this device should be further investigated.

Peri-abutment dermatitis and implant-associated infections

Tjellstrom and Branemark had already noted the occurrence of soft tissue reactions around the BAHS in their first manuscript [38]. They identified hygiene and skin movements as possible reasons to introduce skin reduction surgical approaches. Over the following decades, the abutment designs have been adapted, and skin preservation techniques are currently being endorsed. It seems that we have come full circle. However, skin problems and pain remain relevant issues. In **chapter 6** we demonstrated that BAHS implantation itself leads to up-regulation of various inflammatory markers. Similar results have been shown for BAHS and other applications such as transfemoral bone-anchored prostheses and for dental implants [39–41]. Upregulation of IL-1β, IL-17, IL-6 and TNF-α seem to be associated with inflammation [39–43].

Several mechanisms have been reported to correlate to implant associated infections. Titanium is used for various types of implants. Although titanium seems to be well tolerated by humans, it is not completely inert. Titanium-stimulated macrophages were found to increase the production of IL-1β, IL6, TNF-α and IL-12 [44]. Epithelial cell lines were found

to increase the expression of bone metabolism markers after titanium stimulation [45]. Jacobi *et al.* observed a significantly higher release of IL-1β and TNF-α in response to titanium in patients who had experienced dental implant loss. Genotypes for IL-1, IL1RN and TNFA were found to be correlated to an increased susceptibility for implant loss [46]. *in vitro* titanium particles were found to act synergistically with bacteria to increase cytokine expression [47]. Titanium can modulate the immune system, and inter-individual sensitivity to titanium may contribute to a person's sensitivity to inflammation and bacterial tolerance.

Bacteria can reside on surfaces, such as implants and also tissue surfaces in the form of biofilms, which are more resistant to antibiotic treatment [48,49]. Intra-cellular bacteria can make it even harder to treat biofilms[50]. Moreover, immune responses may be inhibited in the areas adjacent to implants. To date, the best treatment option for biofilm-infected implants is to remove the implant and then re-implant it after adequate antibiotic treatment. Currently, new treatment options for biofilms are being investigated [51,52]. In **chapter 7** we demonstrated that a biofilm can be observed on BAHS. The microbiome contains diverse bacteria, including *Staphylococcus epidermidis* and *Staphylococcus aureus.* Better understanding of the immune alterations near (titanium) implants, of biofilm formation and of susceptibility to peri-implant inflammation could pave the way for more effective treatment options for these types of infections. Implant-related infections are a growing concern in modern medicine. Therefore, not only bone-anchored devices but all implant-associated infections could benefit from new insights into biofilm formation and immune-response mediation associated with implant material.

The treatment modalities for peri-abutment dermatitis can be quite diverse. They may consist of giving advice on local hygiene, applying local ointment, placing healing caps, prescribing systemic antibiotics, performing soft-tissue revision surgery and temporarily removing an abutment. Although each of these options may be effective, there have not been any studies investigating their effectiveness or comparing the treatments. In the absence of certainty, we advise every subject to use a "toothbrush" for local hygiene every night, although there is no proof of the need to do so. The evidence of **chapter 7** suggests that the Terra-Cortril ointment (Pfizer, New York, USA) we often use at MUMC+ is not effective against several bacteria commonly found on BAHS. Alternative treatments might be more effective. Future studies looking into the effectiveness of treatment options could provide us with more evidence-based treatment regimens.

Rehabilitation options and patient characteristics

In recent years, diverse treatment options have become available for several types of hearing loss with overlapping indications. The Air Conduction Hearing Aid is still the first choice for most types of hearing loss. For Bone Conduction, two brands of percutaneous (skin penetration) BAHS are available. Softband or skin patch mounted BAHS sound processors should be considered as possible treatment options as well. It is possible to treat some types of hearing loss with active implants located under the skin, such as the Vibrant Sound Bridge and the Bone Conduction Implant [53]. Alternatively, BAHA Attract, by which a magnet is placed under the skin, has become available [28].

For each of these devices several aspects are important to assess suitability. Many of these devices use magnets, resulting in an inability to perform a MRI of the entire brains post-implantation. In the current health care system, one can expect to undergo a MRI once in life as a minimum. With increasing age, hearing function will deteriorate. One can expect that more powerful devices are necessary over time in many patients. In skin penetration BAHS, a subject has the opportunity to upgrade the device every couple of years allowing easy access to the most powerful devices. Partially or fully implanted devices lack this advantage. Full upgrades require surgical interventions. Amplification range, MRI compatibility and the ease of full updates should be considered in device choice.

Even if all devices are suitable, several issues should be addressed as well. One is that health care providers are unfamiliar with all available options. This can lead to arbitrary discussion making due to a lack of knowledge on suitable treatment options. Another issue is costs. The fact that resources are limited underscores the need for high-quality research to evaluate the extent to which patients could benefit from each solution, as weighed against the respective cost.

Some users may even use different devices over time. Teenagers and young adults may place more value on esthetics, whereas young children and older adults may give priority to optimal hearing results. In conventional air conduction hearing aids, the users often start with a device that is not visible in the ear and then, in time, choose a stronger but larger device. A similar tendency may be seen in bone conduction devices. Before these issues can be addressed, however, it is necessary to have validated outcome measurements that can differentiate between treatment effects in overall quality of life. The quality-of-life questionnaires EQ-5D[54] seem to lack this capability. The HUI-III questionnaire might not have the necessary sensitivity to fully grasp the effect of BAHS on quality of life as it only contains a limited number of questions relating to hearing.

Of particular interest is the Single-Sided Deafness (SSD) population. In these patients, a cochlear implant has been shown to be a possible treatment option as well as BAHS [55,56]. The results for long-term usage of bone anchored devices are relatively low [57]. Knowing more about the medical and personality characteristics of the satisfied versus the dissatisfied users may help us identify the key factors that contribute to the successful use of a device. This knowledge could help improve the user's satisfaction, encourage presurgical consultation and lead to the adaptation of features of the device.

Expected future output

The combination of skin movement analyses, IS-pro™ and cytokine data might produce new insights that would lead to further understanding of the mechanisms related to peri-abutment dermatitis and possibly to percutaneous infections in general. Moreover, given that high-quality photographs were obtained during each subject's visit, the clinical data, skin movements, IS-pro™, cytokines and photographs can be joined in a database that would serve to assess the validity of the Holgers Index [58], IPS scale or possibly an adapted version[24]. The two-year follow-up data, which will provide more information on long-term outcomes of treatment with MIPS and clinical outcomes associated with peri-abutment dermatitis [6].

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Samenvatting

In 1977 werden de eerste botverankerde hoortoestellen ofwel *Bone Conduction Devices* (BCD) geïntroduceerd. Dit hoortoestel wordt achter het oor geïmplanteerd in de schedel. Bovenop het implantaat wordt een abutment geschroefd dat vervolgens door de huid komt. Op het abutment kan een processor worden geplaatst die geluid opvangt en dit in de vorm van trillingen doorgeeft aan het implantaat. Deze trillingen worden omgezet in geluidssensaties in de cochlea (het binnenoor). BCDs worden geïndiceerd bij mensen met een geleidingsverlies, een gemengd gehoorverlies en mensen die enkelzijdig doof zijn.

Deel I

Sinds de introductie van het BCD zijn er verschillende veranderingen geweest. De chirurgische technieken zijn aangepast waarbij de laatste jaren vooral gestreefd wordt naar een minimaal invasieve ingreep met zo min mogelijk weefselschade. In **hoofdstuk 2** is het ontwerp van een multicentrum gerandomiseerde studie beschreven. Deze studie vergelijkt de lineaire incisie techniek met weefselbehoud met een nieuwe punch-only techniek. Deze punch-only techniek is ontworpen door Oticon Medical AB (Askim, Zweden) als gestandaardiseerde techniek met daarbij een specifiek chirurgisch materiaal waaronder nieuw ontworpen boortjes. De studie is in 2013 begonnen met inclusie in Maastricht University Medical Center + (MUMC+) en vervolgens uitgebreid in 2014 in Medisch Centrum Leeuwarden en Ziekenhuis Groep Twente.

In totaal zijn er 64 patiënten geïncludeerd voor deze studie. Na drie maanden follow-up zagen we geen verschillen in de mate van ontsteking (9.1% MIPS vs 16.7% lineaire incisie, p=0.37) of pijnklachten. Wel zagen we bij de MIPS techniek dat er minder gevoelloosheid naast het implantaat was, betere esthetische resultaten en kortere operatietijden. We vonden een trend voor verhoogd implantaat verlies in de MIPS-groep in vergelijking met de lineaire incisie groep. Resonantie Frequentie Analyse (RFA), een veronderstelde maat voor implantaat stabiliteit was lager in de MIPS-groep. Dit verschil was klein en de klinische relevantie hiervan is onbekend. Een uitgebreide rapportage van de resultaten staat beschreven in **hoofdstuk 3**. Hoewel een aantal uitkomsten bij MIPS beter zijn, is implantaat verlies een factor die verder onderzocht moet worden.

Sinds 1992 zijn er in het MUMC+ meer dan 550 eerste BCD-implantaten geplaatst. In **hoofdstuk 4** wordt de follow-up van deze implantaten beschreven. Een analyse toonde aan dat mannelijk geslacht, leeftijd onder de 18 en het hebben van een BCD-implantaat voor een tweede keer een risicofactor zijn voor implantaat verlies. In het gehele cohort werd gezien dat de kansen op implantaat verlies hoger waren voor kortere 3-mm implantaten. Aangezien deze implantaten voor het merendeel in jongere patiënten worden geplaatst, zou ook de jongere leeftijd en niet de implantaatlengte een verklaring hiervoor kunnen zijn. De laatste jaren zijn de implantaten aangepast waarbij bredere implantaten zijn geïntroduceerd. In het algemeen zagen we dat de overlevingscijfers na 1 en 5 jaar hoog waren. In overeenstemming met voorgaande literatuur kunnen bepaalde individuen een verhoogde

kans op verlies hebben. De biologische en gedragsmatige eigenschappen die hierbij een rol zouden kunnen spelen zouden verder onderzocht moeten worden.

Deel II

Zoals eerder beschreven werd er een trend naar verhoogd implantaat verliezen gezien in de MIPS-groep na 3 maanden opvolging. Bij het ontwerp van de MIPS-procedure zijn er een aantal wijzigingen doorgevoerd ten opzichte van de lineaire incisie techniek. Zo is de MIPS-procedure een punch-only procedure waarbij de chirurg niet ziet of het implantaat volledig geplaatst is. Daarbij kunnen de implantaten in een schedelnaad worden geplaatst. Beide factoren zouden mogelijkerwijs kunnen resulteren in een verhoogd implantaat verlies risico. In **hoofdstuk 5** is het gebruik van een Cone Beam CT (CBCT) beschreven. We hebben een aantal implantaten in kunstbot geplaatst om ze vervolgens te scannen. De resultaten van deze *in vitro* testen lieten zien dat we met hoge nauwkeurigheid een implantaat dat volledig in het bot zit kunnen onderscheiden van een implantaat dat niet volledig in het bot zit. Ter controle hebben wij dezelfde techniek toegepast op een aantal patiënten waarvan we twijfelden of het implantaat volledig in het bot zat. In één geval zagen we dat het implantaat schuin in het bot zat. Toevalligerwijze is dit implantaat tijdens follow-up verloren gegaan. Ook vonden we dat RFA-waardes in het bot werden beinvloed door zowel implantatiediepte, abutment lengte en botdichtheid. Deze studie geeft aan dat we CBCT zouden kunnen gebruiken om de implantatiehoek en diepte van BCDs kunnen bepalen. Hoofdzakelijk zou dit een techniek zijn die geschikt is voor onderzoeksdoeleinden.

In **hoofdstuk 6** is de invloed van implantatie op cytokine expressie beschreven. Expressie van interleukine (IL)-1β, IL-6, IL-8, Tumor Necrosis Factor (TNF)-α, IL-17, IL-10, Transforming Growth Factor Bèta (TGF-ß), Macrophage Inflammatory Protein (MIP)-1α, Matrix Metalloprotease (MMP)-9, Tissue Inhibitor of Metalloproteinase(TIMP)-1, Collagen type 1 (COL1α1), Vascular Endothelial Growth Factor (VEGF)-A, basic fibroblast growth factor (FGF-2), Toll Like Receptor (TLR)-2 and TLR-4 is gemeten voor implantatie (baseline) en na 12 weken follow-up middels qRT-PCR. 12 weken na implantatie zagen we dat expressie van IL-1β, IL-8, MMP-9, TIMP-1 en COL1α1 significant hoger was. IL-6 en FGF-2 waren significant verminderd. Binnen patiënten vonden we geen significante verschillen tijdens ontsteking. Een exploratieve analyse toonde aan dat tussen patiënten IL-1β en IL-17 significant hoger was tijdens ontsteking. Bovendien zagen we dat TNF-α significant lager was bij Holgers 1 scores in vergelijking met Holgers 0 scores, een klinische maatstaaf voor ontstekingen. De resultaten van dit hoofdstuk laten zien dat er een verhoogde expressie is van enkele ontstekingsfactoren. Verrassend was de expressie van de ontstekingsfactor IL-6 en vasculaire marker FGF-2 verminderd. De resultaten van deze studie geven aanwijzingen dat na implantatie er een continue activatie is van cytokines in de huid rondom een BCD. Patiëntgebonden factoren als roken en diabetes lijken een rol te spelen in de expressie van enkele van deze cytokines.

In **hoofdstuk 7** zijn de veranderingen in bacteriën op de huid en het botverankerde hoortoestel onderzocht. Middels IS-pro™, een moleculaire techniek, is gekeken naar de aanwezigheid van bacteriën op de huid voor implantatie, na 12 weken follow-up en tijdens ontsteking. De bacteriële profielen van patiënten na implantatie meer op het eigen bacteriële profiel voorafgaande aan de implantatie dan op bacteriële profielen van andere patiënten na implantatie. We vonden geen specifiek abutment profiel. De diversiteit van bacteriën was geen voorspellende maat voor patiënten die een ontsteking hadden of een ontsteking zouden krijgen. Door middel van een model konden patiënten die in de toekomst een ontsteking zouden krijgen worden voorspeld en konden niet ontstoken huid rondom abutments worden onderscheiden van ontstoken huid rondom abutments. *Staphylococcus epidermidis, Streptococcus pneumoniae/mitis* en *Haemophilus parainflenzae* werden veelal gevonden op de huid naast het abutment. Daarnaast werd *Staphylococcus aureus* vaker gevonden tijdens ontsteking van de huid naast het implantaat. In de huidige praktijk wordt vaak Terra-Cortril voorgeschreven. Hoewel dit middel klinisch effectief is, is dit minder effectief tegen de gevonden bacteriën. In de behandeling van patiënten met ontsteking kan hier rekening mee gehouden worden.

Electieve verwijdering van een implantaat is relatief zeldzaam. Bij herhaalde ontstekingsklachten kan het abutment verwijderd worden waarna de huid rustig dicht groeit. In sommige gevallen werkt dit niet en willen patiënten het implantaat volledig verwijderd hebben. In **hoofdstuk 8** beschrijven we een patiënt die persisterende pijnklachten had na abutment verwijdering. Bij deze patiënt was data beschikbaar van micro-CT, histologie, IS-pro™ (bacteriële flora) en klinische data. Histologisch en op micro-CT zagen we een hoge mate van osseointegratie. De IS-pro™ analyse toonde voor abutment verwijdering een grote diversiteit aan bacteriën waaronder *Finegoldia magna*, *Fusobacterium nucleatum*, *Staphylococcus aureus*, *Staphylococcus epidermidis* en *Streptococcus sanguinis*. Dezelfde bacteriën werden 10 maanden later op het implantaat gevonden. *Staphylococcus aureus*, een veelvoorkomende bacterie bij huidontstekingen, was de meest voorkomende bacterie op het implantaat. *Staphylococcus epidermidis* en *Staphylococcus aureus* werden ook in de huid rondom het implantaat gevonden. Daarnaast vonden we aanwijzingen voor bacteriën in het bot rondom het implantaat. Dit case-report toont aan dat de chronische pijn mogelijk veroorzaakt kan worden door een geïnfecteerd implantaat zonder dat er klinisch duidelijke tekenen van ontsteking zijn. Het gaat hier om een enkele waarneming die bevestigd zou moeten worden in toekomstig onderzoek.

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Het BCD-team

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Curriculum Vitea

Tim George Ate Calon was born on 1st of February 1988 in Boxmeer, the Netherlands. He attended Athenaeum pre-university education at RSG 't Rijks in Bergen op Zoom. After, obtaining his Bachelor and Master degree in Medicine at Maastricht University in November 2013. He started his medical career in Internal Medicine at Maasstad Ziekenhuis, Rotterdam. In October 2014, he started his doctoral scientific research under the supervision of professor dr. Robert-Jan Stokroos at the department of Otorhinolaryngology, head and Neck surgery at Maastricht University Medical Center. Since February 2018, he commenced his specialization as resident in Internal Medicine at Catharina Ziekenhuis in Eindhoven.