The percutaneous implant. The effects of design, host site and surgery on the tissue response

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Dedicated to Dima, Feri and Siri

ABSTRACT

This research project focuses on the multiple challenges associated with implants that breach the skin. The role of device design, host site, and surgical approach on tissue response and outcome are evaluated, experimentally and clinically, for both a stoma port and a bone-anchored hearing system (BAHS). Experimental studies implied the opportunity to integrate a soft-tissue-anchored titanium port with skin and intestine. However, the longevity was challenged by the presence of bacteria and mechanical strains [Paper I, II]. Applying a micro- and nanotopography to a bone-anchored implant, enhanced its biomechanical anchorage in comparison with a machined surface. Further it was found that removal torque was associated with the degree of bone-to-implant contact, whereas the implant stability, at retrieval, was correlated with the bone area [Paper III]. Bench tests demonstrated that during osteotomy preparation, the level and distribution of heat is affected by the drill design, and the drilling and irrigation procedure. Provided that the recommended, standard procedure is followed, the absolute temperatures using either a conventional drill system or a guided drill system are below the threshold for thermally induced tissue damage [Paper IV]. In human studies of BAHS, anaerobic bacteria, but not aerobes, were detected in the tissue already prior to installation. In contrast, after the installation of BAHS, both anaerobic and aerobic (predominantly *S. epidermidis* and *S. aureus*) bacteria were detected on the abutment, in the tissue and in the peri-abutment fluid space, at both three and 12 months [Paper V]. The feasibility of a novel, minimally invasive technique for installing BAHS was demonstrated clinically [Paper VI, VII, VIII]. In a randomized clinical trial, skin sensibility and cosmetics were significantly better and, surgery time and skin sagging was significantly reduced compared with the conventional surgical procedure. At three-months, no significant difference in incidence of inflammation was found between the techniques [Paper VIII].

It is concluded that the device design, host site, and surgical approach are important determinants for the tissue response and clinical outcome of percutaneous systems.

Keywords: abutment; bacteria; BAHA; BAHS; biomaterial; bone; bone anchored hearing; bone damage; bone drilling; drill design; drill mechanics; drilling temperature; enterostoma; flapless; guided surgery; hearing loss; heat generation; ileostomy; implant; infection; inflammation; minimally invasive surgery; MIPS; osteotomy; osseointegration; percutaneous; skin; soft tissue; surgery; titanium; tissue response; urostomy

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SAMMANFATTNING

Permanenta implantat används i allt större utsträckning för att behandla olika medicinska tillstånd. Flera av dessa implantat penetrerar hud och andra vävnader. Hudpenetrerande (s.k. perkutana) implantat möjliggör åtkomst till kroppens inre men bryter hudens skyddande barriär vilket medför en ökad risk för komplikationer. Negativa vävnadsreaktioner och infektion utgör viktiga utmaningar. I syfte att förbättra klinisk behandling och resultat behövs ökad kunskap om betydelsen av implantatutformning, lokala vävnadsförhållanden och kirurgisk metodik. Syftet med detta avhandlingsarbete var att förstå hur dessa aspekter påverkar vävnadsreaktioner och patient-relaterade utfallsparametrar. Implantatdesign och kirurgiska metoder utvärderades för två typer av hudpenetrerande implantat, dels ett mjukvävnadsförankrat implantat i syfte att skapa en kontinent stomi, dels ett benförankrat implantat avsett för behandling av hörselnedsättning (BAHS).

Experimentella studier påvisade möjligheten att integrera en perkutan titanport med hud- och tarmvävnad för att åstadkomma en kontinent stomi. Närvaro av bakterier samt mekanisk påfrestning utgjorde stora utmaningar. Resultaten antyder ett behov av ökad kunskap innan klinisk användning [delarbete I, II].

Vid studier av benbildning kring titanimplantat erhölls en bindning mellan ben och implantat (korrelativ ljusmikroskopi och elektronmikroskopi) och en väsentlig ökning av den biomekaniska förankringen med ett laserbehandlat implantat med kombinerad mikro- och nanotopografi jämfört med ett implantat med slät yta. Dessutom påvisades samband mellan graden av benkontakt och urvridningsmoment, respektive mellan mängden ben i implantatets gänga och implantatstabilitet [delarbete III].

En minimalinvasiv kirurgisk metod (MIPS) med tillhörande borrsystem har nyligen utvecklats. Borrning och installation genomförs via ett titthål i mjukvävnaden till skillnad från den konventionella metoden där mjukvävnaden viks undan och benytan exponeras. När ett benförankrat implantat opereras in är det av vikt att bibehålla bencellernas viabilitet trots värmeutveckling vid borrning. Omfattande tester i artificiellt ben visade att värmeutveckling och fördelning av värme i omgivande ben påverkas av borrens konstruktion, hur borrningen genomförs och hur kylvätska appliceras. Under förutsättning att det rekommenderade standardprotokollet för borrning efterföljs, visades att de absoluta temperaturerna för både det konventionella systemet och MIPS var lägre än tröskeln för termiskt inducerad vävnadsskada [delarbete IV].

I en prospektiv klinisk studie, var detektionen av huvudsakligen anaeroba bakterier i mjukvävnaden innan implantation av BAHS ett oväntat och viktigt fynd. Efter BAHS-implantation däremot, detekterades både aeroba och anaeroba bakterier på den hudpenetrerande distansen, i omgivande distansficka samt i omgivande mjukvävnad efter både 3 och 12 månader. Kolonisation av *Staphyloccus epidermidis* (11/12 patienter) samt *Staphylococcus aureus* (5/12 patienter) var vanligt förekommande [delarbete V].

Säkerheten och effektiviteten av den nya kirurgiska metoden MIPS för installation av BAHS demonstrerades dels i en klinisk multicenter-utvärdering [delarbete VI], dels i en kontrollerad randomiserad multicenterstudie av patienter med konduktiv eller kombinerad konduktiv och sensorisk hörselnedsättning [delarbete VII, VIII]. Signifikant mindre förlust av hudkänsel, förbättrat kosmetiskt resultat och reducerad operationstid visades med MIPS jämfört med det konventionella kirurgiska förfarandet. Efter tre månader påvisades ingen signifikant skillnad mellan de kirurgiska teknikerna avseende förekomst av inflammation i omgivande mjukvävnad [delarbete VII, VIII].

Sammanfattningsvis visar föreliggande avhandling att design, vävnadslokal och kirurgisk metod utgör viktiga faktorer för inläkning och lyckat behandlingsresultat med hudpenetrerande implantatsystem.

LIST OF PAPERS

This thesis is based on the following eight studies, referred to in the text by their Roman numerals.

I. Johansson ML, Thomsen P, Hultén L, Halvorsen PS, Fosse E, Edwin B. Integration between a percutaneous implant and the porcine small bowel. *Journal of Biomedical Materials Research Part B: Applied Biomaterials.* 2011;98(1):101-9.

II. Johansson ML, Hultén L, Peeker R, Jonson O, Thomsen P, Edwin B. Achieving stoma continence with an ileal pouch and a percutaneous implant. *In manuscript*

III. Shah FA[#], Johansson ML[#], Omar O, Simonsson H, Palmquist A, Thomsen P. Laser-Modified Surface Enhances Osseointegration and Biomechanical Anchorage of Commercially Pure Titanium Implants for Bone-Anchored Hearing Systems. *PLoS One.* 2016;11(6):e0157504. [#]Contributed equally.

IV. Johansson ML, Eriksson, T, Omar O. The development and ex vivo evaluation of a novel drill system for bone conduction hearing implants. *In manuscript.*

V. Trobos M[#], Johansson ML[#], Jonhede S, Simonsson H, Hoffman M, Omar O, Thomsen P, Hultcrantz M. The clinical outcome and microbiological profile of bone anchored hearing systems (BAHS) with different abutment topographies – A prospective pilot study *Submitted for publication*. [#]Contributed equally.

VI. Johansson ML, Stokroos RJ, Banga R, Hol MK, Mylanus EA, Savage Jones H, Tysome JR, Vannucchi P, Hof JR, Brunings JW, van Tongeren J, Lutgert RW, Banerjee A, Windfuhr JP, Caruso A, Giannuzzi AL, Bordin S, Hanif J, Schart-Morén N, Singam S, Jonhede S, Holmberg M, Cremers CW, Hultcrantz M. Short-term results from seventy-six patients receiving a bone-anchored hearing implant installed with a novel minimally invasive surgery technique. *Clinical Otolaryngology.* 2017;42(5):1043-1048.

VII. Calon TG, van Hoof M, van den Berge H, de Bruijn AJ, van Tongeren J, Hof JR, Brunings JW, Jonhede S, Anteunis LJ, Janssen M, Joore MA, Holmberg M, Johansson ML, Stokroos RJ. 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. *Trials.* 2016;17(1):540.

VIII. Calon TGA[#], Johansson ML[#], de Bruijn AJG, van den Berge H, Wagenaar M, Eichorn E, Janssen AML, Hof JR, Brunings JW, Joore MA, van Tongeren J, Jonhede S, Holmberg M, Stokroos RJ. Minimally Invasive Ponto Surgery versus the Linear incision technique with soft tissue preservation for Bone Conduction Hearing Implants: A multicentre randomized controlled trial. *Otology & Neurotology. Accepted for publication.* 2017. [#]Contributed equally.

Additional publications not included in this thesis:

- Tysome JR, Hill-Feltham P, Hodgetts WE, McKinnon BJ, Monksfield P, Sockalingham R, Johansson ML, Snik AF. The Auditory Rehabilitation Outcomes Network: an international initiative to develop core sets of patient-centred outcome measures to assess interventions for hearing loss. *Clinical Otolaryngology* 2015;40(6): 512-515.

- Calon TG, Johansson ML, van den Burg EL, Janssen AML, van Hoof M, Stokroos RJ. The Use of Cone Beam Computed Tomography in Assessing the Insertion of Bone Conduction Hearing Implants. *Frontiers in Surgery.* 2017;4(38): 38.

- Kruyt IJ, Nelissen RC, Johansson ML, Mylanus EAM, Hol MKS. The IPS-scale: A new soft tissue assessment scale for percutaneous and transcutaneous implants for bone conduction devices. *Clinical Otolaryngology* 2017;42(6): 1410-1413.

CONTENTS

1 INTRODUCTION

On June 11th 1892, the esteemed US surgeon John Benjamin Murphy decided *"…to perform cholecystoenterostomy by means of my anastomosis button, which I had used for the first time on a dog six days previous."* [1]. To facilitate the joining of the gallbladder to the intestine, Murphy devised two rings from brass that when joined and locked together, achieved a rapid anastomosis of the two tissue segments. Within a week, the metal sphere was then ''passed'', leaving the anastomosis intact. This device, the anastomosis button is known as ''the Murphy Button''. After performing the first cholecystoenterostomy on a 35-year-old female, Murphy wrote; *"Time from opening of the peritoneum until the closing of same, eleven minutes."* In Murphy's hands, the use of the button reduced the operating time for this anastomosis tenfold. Translating the idea to design and surgical approach, via experimental work and finally to the clinical use of an implant within a few weeks is not quite the way we work today, although that does not diminish Dr Murphy's ingenuity. Even though we would not accept his approach today, his invention is still used, albeit with different material and designs. However, the process he facilitated is not that far from what we employ today.

Surgically inserted biomaterials and implants to treat medical conditions have been used increasingly, creating major benefits for patients and society. Nevertheless, adverse tissue reactions and infection still pose major challenges. An increased knowledge of biomaterials, tissue responses and outcomes is therefore needed to produce improved products and surgical approaches. Multiple interrelated factors influence the short- and long-term outcome, the longevity and ultimately the treatment benefit following the surgical installation of an artificial prosthesis.

Taking a holistic view, these factors can be attributed to four domains; the device, the surgery, the patient and the maintenance. The device includes all the aspects related to the prosthesis and tools used, material, shape and texture from macro- to nanoscale and its physico-chemical surface characteristics. All these properties influence the host-tissue response. The tissue response following the installation of the device is also influenced by the surgical trauma caused by installing the device in its intended position. The specific anatomical site where the implant is inserted will pose different challenges and set different design criteria. Not only the patient's local tissue status but also the systemic condition will have an impact and factors such as smoking, BMI, diabetes, previous irradiation and age have been shown to influence the tissue response to and outcome for a prosthesis. Finally, a domain that is often overlooked is maintenance. How the device is handled and used on a day-to-day basis by the patient and, in the case of adverse events, how they are treated. In this thesis, some of these domains are addressed.

1.1 Epithelial penetration

A deer's antler is a naturally occurring skin-penetrating formation that has served as an important inspiration for manmade transcutaneous prostheses [2, 3]. The antler is firmly anchored to the skull bone and when fully grown, the protective skin is shed leaving a bare antler penetrating all layers of the skin. In this transient transcutaneous phase, the porous pedicle bone is integrated with the surrounding subepithelial dermal tissue via collagen fibres emanating from the pores and spanning the dermal soft tissue-pedicle interface [3, 4]. Superficially, an epithelial layer, which is interfaced with the pedicle bone without signs of downgrowth into the underlying dermal tissue, can be seen [4]. The tight seal between the sub-epithelial dermal tissues and the pedicle bone appears to present a physical barrier to epithelial cell migration (epithelial downgrowth), as well as the invasion of debris and microbes into the interface.

Another natural situation where the epithelium is breached, in this case permanently, is the tooth. The root is attached to the bone by the periodontal ligament, creating a firm mechanical anchorage. The gingival tissue surrounding the neck of the tooth consists of a mucous membrane covering the subepithelial connective tissue. Inferior to the gingival sulcus, the gingival connective tissue and the tooth surface are interfaced by the junctional epithelium. The junctional epithelium is characterised by rapid turnover [5] and in contrast to most other epithelia, it is nonkeratinised. Instead a basement membrane is interposed to the gingival connective tissue and a basal lamina forms part of the interfacial matrix between the tooth-facing junctional epithelial cells and the tooth surface [6]. This structural and functional adaptation of the interface maintains the integrity against the constant bacterial, chemical and mechanical challenges.

1.2 Skin

The skin is one of the largest organs in the body in terms of surface area and weight. The skin consists of three main layers: the epidermis, the dermis and the hypodermis (Figure 1). The primary function of the skin is to provide protection from mechanical impact and pressure, variations in temperature, micro-organisms, radiation and chemicals. The skin also regulates several aspects of physiology, including body temperature via sweat and hair and changes in peripheral circulation and fluid balance via sweat. It also acts as a reservoir for the synthesis of Vitamin D. Further, the skin contains an extensive network of nerve cells that detect and relay changes in the environment.

The epidermis constitutes the surface in contact with the external environment and it is a keratinised, stratified, squamous epithelium. Keratinocytes, the main cellular component of the epidermis, are tightly connected to one another by hemidesmosomes. They arise in the deeper part of the epidermis and are pushed up by the production of new cells beneath them. Their lifespan is about four weeks. By the time they approach the skin surface, they are dead cells and are shed from the body [7]. Keratinocytes, as well as melanocytes and dendritic cells within the epidermis, have immunological functions.

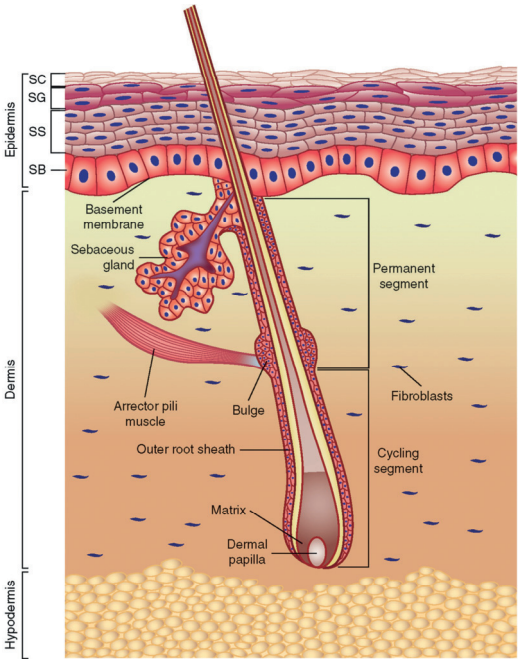

Figure 1 *Anatomy of the skin. Skin is composed of three layers, starting with the outermost layer: the epidermis, dermis, and hypodermis. The epidermis is a stratified squamous epithelium that is divided into four layers, starting with the outermost layer: stratum corneum (SC), stratum granulosum (SG), stratum spinosum (SS), and stratum basale (SB). The outer root sheath of the hair follicle is contiguous with the basal epidermal layer. © 2009 David J. Wong and Howard Y. Chang [8]. Creative Commons Attribution 3.0 Unported License.*

The dermis is composed of cells typically found in connective tissue, such as fibroblasts, mast cells, macrophages, lymphocytes and endothelial cells. The primary cellular component is the fibroblast, which is of mesenchymal origin. In the deeper layer of the dermis, thick bundles of interlacing collagen and elastic fibres are oriented mostly parallel to the skin. The dermis is not only a support substrate for the epidermis, but also a complex structure that has important signalling communication with the epidermis which is vital to the homeostasis of the skin. Fibroblasts produce and organise extracellular matrix and communicate with each other and with the epidermis through various signalling pathways [9].

1.3 Bone

Bone is a complex, vascularised, cellular and highly mineralised connective tissue. Its main functions are to provide mechanical support and framework, permit locomotion, anchor the muscles, protect vital organs and act as a metabolic reservoir of mineral salts. One principal component of bone is the organic matrix, composed predominantly of Type I collagen, which provides resistance to tensile forces. The second main component is calcium phosphate, mainly hydroxyapatite crystals, that adds compressive strength to the framework [10]. The basic unit of bone is the osteon: each osteon comprises concentric lamellae of compact bone surrounding a central canal (Haversian canal) which contains blood vessels. Morphologically, bone is divided into cortical and trabecular bone. Cortical bone is compact or dense and forms the hard, outer layer of bones while trabecular bone makes up the inner layer of the bone and has a spongy structure. Bone can also be classified as long bones, like the tibia and femur, flat bones, like skull bone, and irregular bone such as the hip bone. The internal and external surfaces are lined with cellular layers called endosteum and periosteum respectively [10].

Bone is a dynamic organ that undergoes significant turnover compared with other organs in the body. Complex intercellular signalling, between the osteoprogenitor cells and mature bone cells, regulates and balances activities of bone cells during remodelling and growth [11]. Three different mature bone cells are involved in this regulation; osteoblasts, osteocytes and osteoclasts. The co-ordinated activity of osteoclasts and osteoblasts is important in maintaining bone structure. Bone is formed by osteoblasts which are derived from pluripotent mesenchymal stem cells. Their primary function is the synthesis and mineralisation of osteoid and the organic matrix. Osteoclasts, bone-resorbing cells, are large, multinucleated cells derived from the monocyte/macrophage lineage. Their function is the localised degradation of bone matrix and mineral during bone resorption. Osteocytes are embedded in lacunae (pits) in the bone matrix and are formed when osteoblasts become trapped inside the matrix. They are interconnected via cytoplasmic extensions running through a canalicular (canal) network. Osteocytes act as sensors and convert the stimuli of mechanical loading into biochemical signals [11]. Bone remodelling is performed by osteoblasts and osteoclasts working in tandem organised into a “cutting cone” with osteoclastic resorption at one end and osteoid formation at the other. One tenth of the bone volume undergoes remodelling every year.

1.4 Osseointegration

Osseointegration is the outcome of bone tissue healing and adaptation to an implant. In this biological process, the anchorage of the prosthesis is achieved by the formation of bone tissue around the implant, without an intermediate layer of fibrous tissue [12]. An advocated definition of osseointegration is therefore "*…a direct – on light microscopic level – contact between living bone and implant.*" [13]. This direct contact between bone and implant (bone-to-implant contact, BIC) is however never 100%. In parallel with the introduction of new processing and analytical techniques relating to the interface between material and tissue [14], new insights into the ultrastructure of this interface [15] and the role of the osteocyte [16] have emerged. For a further understanding of the mechanisms of osseointegration, additional knowledge of the relationship between implant design, bone-implant structure and composition and subsequent function is needed.

Dental implants, pioneered by P.I. Brånemark in the 1960s [12], are an example of devices permanently penetrating the soft tissue while being anchored in the bone. The treatment of edentulous patients with dental implants is now common practice in modern dentistry, with survival rates of over 94 % at 10 years [17]. Their success relies in part on osseointegration. A cascade of biological events, including initial inflammation, bone formation and bone remodelling, is involved in achieving osseointegration [18].

At present, many implant-based treatments in clinical use, such as the bone-anchored hearing aid for the treatment of hearing loss [19], the retention of maxillofacial prostheses after craniofacial reconstruction [20] and bone anchored implants for the attachment of limb prostheses [21], rely on osseointegration for the anchorage and stabilisation of the prosthesis. Extensive experimental and clinical work suggests that the biological response and osseointegration can be modulated by the physico-chemical properties of the implant surface as well as its bulk properties in different length scales [18, 22-25]. Numerous surface modifications, including turned, blasted, acid-etched, porous-sintered, oxidised, plasma-sprayed, laser-modified, hydroxyapatite-coated surfaces, highly hydrophilic or a combination of these procedures, have been developed and are currently used with the aim of enhancing clinical performance [26]. Apart from the obvious commercial push, the driver has also been to reduce time until loading. Moreover, although the success rate of oral and maxillofacial implants is generally high, some patient groups run an increased risk of losing the implant [27, 28]. Factors such as low or high age [29-31], bone quality and quantity [32], smoking status [33, 34], systemic diseases [35] and previous radiotherapy [36] have been shown to be

potential risk factors. All these factors necessitate greater implant stability in both the short and the long term.

Implant surface modification is a main strategy that can optimise the surface properties of an implant and promote its integration in the recipient bone. The physical (e.g. topography) and chemical (e.g. oxide thickness) properties of an implant surface play a critical role in modulating the tissue response [37]. Originally, Brånemark implants had machined (turned) surfaces. Turned surfaces are those originally formed by the machining procedure of the titanium bar and they are relatively smooth surfaces. The addition of micron-level topography to conventionally machined surfaces greatly enlarges the surface area and improves the mechanical interlocking between the implant surface and bone. Moderately roughened surfaces permit higher stability and better clinical results [22, 38, 39]. It has been suggested that a combination of surface roughness at different distinct length scales (e.g. micron, submicron and nano) superimposed on one another benefits bone-implant bonding, particularly if these functionally graded surfaces mimic the hierarchical architecture of natural bone [40, 41]. By themselves, nanostructures on a surface could stimulate the osteogenic differentiation of mesenchymal stem cells *in vitro* [42]. Moreover, implant surfaces that incorporate well-defined nano-topography stimulate osseointegration *in vivo* [43].

Following this concept, many procedures were developed to roughen implant surfaces; however, one potential drawback was empirically observed: a suspected increase in the incidence of peri-implantitis when compared with implants with turned surfaces [44]. Peri-implantitis can be defined as “a site specific, plaque-induced infection with progressive loss of the bone supporting a functioning implant” [45]. In one retrospective evaluation, this chronic infection affects at least 16% of people rehabilitated with dental implants with turned surfaces after nine to 14 years in function [46]. Even though the reported frequency of peri-implantitis varies, turned implant surfaces are considered to be less susceptible to peri-implantitis. There is a potential risk that implants with roughened surfaces could run a higher risk of causing peri-implantitis.

1.5 Wound healing and foreign body reaction

Intact epithelia constitute a barrier to protect the body from injury and invading micro-organisms. Once that barrier is breached, protective inflammatory and immune responses are activated. Inflammation is a fast, non-specific and often local response, whereas the immune response is highly specific and takes longer to develop. The wound-healing process in response to injury involves discrete yet inter-related and overlapping stages; (i) coagulation, (ii) inflammation, (iii) proliferation and (iv) remodelling [47]. After an initial

phase of haemostasis, the inflammatory phase prevents infection by neutrophils (polymorphonuclear cells, PMNs), macrophages, antibodies and growth factors being recruited to the site. It is at this stage that the characteristic signs of inflammation can be seen; erythema, heat, oedema and pain. In the following proliferative or granulation phase, angiogenesis is initiated and cells like macrophages, myofibroblasts and fibroblasts enter the site. The wound is rebuilt with new tissue made up of collagen and extracellular matrix, the granulation tissue. Cells such as macrophages and fibroblasts are involved in this phase. Eventually this leads to the closure of the wound (re-epithelisation) and a remodelling phase with the formation of scar tissue [47].

In a similar manner, the healing process after the implantation of a biomaterial involves a cascade of events and an interaction between many factors. However, due to the temporary or continuous presence of the implant, the sequence of events follows another pathway, eventually resulting in fibrous encapsulation in a process called foreign-body reaction (FBR) [48]. The overall goal of the inflammatory response and wound healing is to restore the body's homeostasis. With the presence of a biomaterial, there are four main types of possible resolution. (i) Extrusion: if the implant is in contact with epithelial tissue (top layer of skin), the material can be forced out of the body. (ii) Resorption: in the case of a biodegradable material (depending on the degradation rate), a fibrous capsule will or will not be formed. (iii) Integration: the close approximation of host tissue to the implant, with no intervening fibrous capsule (e.g. titanium in bone). (iv) Encapsulation: the formation of a fibrous capsule around the implant. After the injury inflicted by the insertion of the biomaterial, the following temporal sequence of events is: (i) protein adsorption, (ii) acute inflammation, (iii) chronic inflammation, (iv) foreign-body reaction, granulation formation and, finally, (v) the fibrous encapsulation of the biomaterial [49]. These processes are mediated and orchestrated by cytokines, chemokines and matrix metalloproteinases (MMPs) [50]. Many general mechanisms of the host response to a biomaterial have been elucidated. Nevertheless, in this complex and interrelated scenario, our basic understanding of the molecular interactions is still limited. Additionally, these processes can be influenced and mediated through the implant design, surface properties, localisation, host-bed status, surgical technique and mechanical loading.

After an injury and the insertion of the biomaterial in the living tissue, proteins from the blood plasma are immediately adsorbed to the surface to form a provisional protein matrix. The composition of this matrix is known to be dependent on the physico-chemical properties of the material [51]. Acute inflammation is of relatively short duration, lasting from minutes to days, depending on the extent of the injury. The tissue damage during surgery

appears to be the main trigger for the activation of the innate immune response by the infiltration of predominantly polymorphonuclear leucocytes (PMN) and mast cells. Growth factors such as interleukin 8 (IL-8), matrix metalloproteinases (MMPs), macrophage inflammatory protein 1α (MIP-1α) and tumour necrosis factor-α (TNF-α) are involved in the regulation and increased recruitment of leucocytes and monocytes.

The chronic inflammatory phase is characterised by the presence of mononuclear cells (lymphocytes and macrophages), indicating that the material has triggered an acquired immune response. The monocyte-derived macrophages have been suggested as the most important cell in the chronic inflammatory phase because of the large number of biologically active products they can produce [52, 53]. This also applies to the sub-acute and chronic inflammatory stage associated with biomaterials [48, 54, 55]. However, there are indications that the lymphocyte and its interaction with the macrophage also play a significant role [56]. The initiation of wound healing is marked by the arrival of fibroblasts and endothelial cells, recruited by factors secreted by the macrophages. These cells proliferate to form granulation tissue. At this stage, macrophages fuse to form multinucleated foreign-body giant cells (FBGC) on the surface and in the vicinity of the biomaterial, a process that is currently incompletely understood [53]. The formation of FBGCs is the hallmark of the foreign-body reaction and constitutes what separates the healing process from a normal wound-healing process. The presence of FBGCs at the biomaterial surface is considered to be undesirable, as, since in the long term they are the main source of bioreactive agents like reactive oxygen species (ROS), degradative enzymes and acids, which may lead to the biodegradation of the implanted material and, ultimately, device failure [56]. Macrophages and FBGCs adhering to an implant surface have reduced bactericidal capacity, since the cells are exhausted by trying to phagocytise the foreign body [57]. As a result, it is therefore believed that the adhesion of FBGC to the implant surface is an undesirable situation. The ideal outcome after biomaterial implantation would be the full restoration of normal tissue and function, just like after wound healing. With only a few exceptions, fibrosis and fibrous encapsulation of the prosthesis is the final condition following this cascade of tissue responses to a biomaterial.

For percutaneous implants, there is often an absence of fibrous encapsulation and the system cannot be fully encapsulated [58]. Moreover, the downgrowth of non-keratinised epidermis is typically present in an attempt by the body to extrude the device [59]. This also appears to keep the tissue close to the skin-penetrating abutment in a chronic state of inflammation [59]. It is less well known how the inflammation around abutments develops over time and whether it is affected by surgical techniques.

1.6 Skin, inflammation and infection

The skin is the primary barrier to micro-organisms. The surface is colonised by micro-organisms, including bacteria, fungi and viruses, most of which are harmless or even beneficial to their host. There are also data suggesting that the microbiota extends within the dermis [60]. As a result, the deeper layer of the skin, which was previously thought to be sterile, may be colonised by bacteria. Skin microbiome studies have shown the immense diversity of bacteria residing on human skin with high interindividual variability [61, 62]. Factors specific to the host, such as age, location and gender, have been shown to influence the microbial flora of the skin [63, 64]. The lowest bacterial diversity was found in sebaceous sites like the retro-auricular crease (behind the ear) with 15 phylotypes [61, 62]. Further, the environmental conditions, humidity and temperature, have been shown to influence the quantity and distribution of bacteria on the skin [65]. *Propionibacterium* spp., *Staphylococcus* spp. and *Corynebacterium* spp. predominate in sebaceous sites in the skin in culture-based [66] and genomic [61] studies.

In an assessment of the prevalence of hospital-acquired central line-associated bloodstream infections, catheter-associated urinary tract infections, ventilator-associated pneumonias and surgical-site infections, the most common pathogens included *Escherichia coli* (15%), *Staphylococcus aureus* (12%), Klebsiella species (8%) and coagulase-negative staphylococci (8%) [67]. In general, the proportion of isolates with common resistance phenotypes was higher among device-associated infections compared with surgical-site infections. Among the pathogens resulting in surgical-site infection, *S. aureus* was the most commonly reported pathogen overall (21%), followed by *E. coli* (14%), coagulase-negative staphylococci (8%), and *Enterococcus faecalis* (8%) [67].

1.6.1 Biofilm and biomaterial-associated infection

Foreign bodies inserted in living tissue are predisposed to infection, as they provide surfaces for biofilm formation. Biofilms are communities of microorganisms that grow attached to a surface or interphase and embedded in a self-produced extracellular matrix [68]. In a classical experiment from 1957, Elek and Cohen demonstrated the infection-enhancing effect of biomaterials where the minimum abscess-producing inoculum was >10,000-times lower in the presence of a biomaterial [69]. In contrast to infections caused by planktonic bacteria, biofilm infections have the following characteristics: (i) bacteria adhere to a surface, (ii) bacterial clusters are encased in an extracellular polymeric matrix, (iii) infection is confined to a local site, (iv) adherent bacteria may be undetectable using routine diagnostic procedures, (v)

infection generally persists despite the susceptibility of planktonic bacteria to the antimicrobial agent and (vi) host defences are unable to eradicate micro-organisms, i.e. a spontaneous cure does not occur [70]. Many types of chronic infection, such as otitis media, cystic fibrosis pneumonia, post-traumatic osteomyelitis and chronic wounds, are caused by bacterial biofilms [71].

A biomaterial-associated infection (BAI) is typically caused by micro-organisms that grow in biofilms [72, 73]. Development occurs stepwise, beginning with the adherence of bacteria to the proteins adsorbed on the implant surface, followed by their proliferation and differentiation and culminating in their dispersion. Following adhesion, the secretion of a sticky matrix of self-produced extracellular polymeric substances, consisting of polysaccharides, proteins and extracellular DNA, is essential for the formation and maintenance of the biofilm. The matrix gives mechanical stability and enhances the distribution of nutrients within the biofilm [74]. Furthermore, the matrix holds cells close to each other, allowing cell-to-cell communication using so-called quorum sensing, allowing the bacteria to synchronise the gene expression of the group and thus act in unison [75]. Biofilms are able to propagate through the detachment of small or large clumps of cells, or by a type of "seeding dispersal" that releases individual cells. Either type of detachment allows bacteria to attach to a surface or to a biofilm downstream of the original community. BAIs resist host defence and most antimicrobial agents through four major resistance mechanisms; an altered microenvironment within the biofilm, persistent cells, slow penetration of the antimicrobial and reduced metabolic activity of the bacteria [76].

1.7 Challenges with percutaneous implants

1.7.1 Breaching of the skin barrier

Percutaneous implants are being used increasingly in many applications for the treatment of disorders, the retention of prostheses and the administration of substances and energy through the skin [20, 77]. Despite attempts to increase the robustness of the solutions, infection remains the major complication and is associated with considerable cost, morbidity and even mortality [21, 77-83] (Table 1). Like all biomaterials, the main factors in this increased susceptibility to infection are the local impairment of the host immune defence and the formation of biofilm on the device [84]. For percutaneous implants, breaching the skin constitutes an additional load on the system, with the continuous risk of microbe invasion and debris at the junction between tissue, implant and external environment. Other failure modes, alone or in combination, for percutaneous devices have been encountered, including the formation of a

sinus tract (marsupialisation), mechanically induced inflammation (avulsion) and epidermal downgrowth (permigration) [85].

1.7.2 Soft-tissue attachment

At present, the prevailing idea is that a skin seal around the abutment is preferable. This is based on the logic that it could disable invasion by pathogens. To achieve successful soft-tissue integration, fibroblasts must win the "race for the surface" against micro-organisms on the implant surface [72, 86]. The concept is supported by the idea that similar integration is also found in our own body around our teeth, for example, or in nature in horn-bearing animals [4]. This notion seems sound and has received support from *in-vitro* and some *in vivo* animal studies. However, as of today, there is no *in vivo* human scientific evidence supporting the belief that tighter adherence of the skin to an implant surface would result in a more desirable soft-tissue outcome. Recently, a hydroxyapatite-coated abutment was evaluated in a sheep model demonstrating a significant reduction in pocket depth and epithelial downgrowth, as well as improved soft-tissue integration compared with conventional titanium abutments [87]. This contradicts previous findings where fibrous encapsulation was demonstrated for hydroxyapatite percutaneous implants [88]. It should be noted that the implant mechanical design and animal model, as well as the host site, were different in these experimental studies. However, against the assumption that a tight structural barrier is a prerequisite and proving that infection remains a risk, the translation of these promising experimental results into an improved clinical outcome with respect to inflammation and infection has not been seen, at least not in the short term [89, 90].

1.7.3 Inflammation and infection

Multiple biological processes are elicited around skin-penetrating implants; they include the downgrowth of epithelium, the increased infiltration of inflammatory and immunocompetent cells and the entry of micro-organisms into deeper layers of the skin [59, 91]. However, relatively few studies have investigated the structural and functional barrier of the interface between a percutaneous implant and soft tissue. A common observation around titanium abutments for bone-anchored hearing systems is the presence of the downgrowth of non-keratinised epidermis but the lack of fibrous encapsulation [59]. Further, there is a constant and ongoing inflammatory process. This is corroborated by the findings of accumulations of inflammatory cells close to the implant, regardless of clinical signs [58].

Table 1 *List of percutaneous medical devices and implants (adapted from [77] and [92])*

Device	Anchorage	Implantation	Infection rate (%)	Ref
Blood access device				
Central venous catheter	Soft tissue	Permanent/ temporary	0.5-23	[93]
Heart assist device (drive line infection)	Soft tissue	Permanent/ temporary	26-50	[82, 93]
Haemodialysis dialysis catheter	Soft tissue	Months to years	8-21	[93]
Body cavities access device				
Urinary catheter	Soft tissue	Temporary	3-10 (per day)	[81]
Peritoneal dialysis catheter	Soft tissue	Months to years	3-5	[92]
Long-term gastrostomy	Soft tissue	Long term	5-25	[94]
Percutaneous access port	Soft tissue	Permanent	2-15	[80]
Bone-anchored devices				
Dental implant	Bone	Permanent	5-10	[95]
Limb prosthesis	Bone	Permanent	Skin: 34-66 Deep: 10	[96] [97]
External fixator	Bone	Temporary	Minor: 8-67 Major: 0-4	[83] [98]
Bone-anchored hearing system (BAHS)	Bone	Permanent	1-21	[99]

Staphylococci are commensals of the skin and mucous membrane of humans. *Staphylococcus epidermidis* and *S. aureus* are among the leading causes of hospital acquired infection [71]. Infections due to *S. epidermidis* are typically more subacute or chronic compared with *S. aureus* which causes more acute infections [100]. Osteomyelitis in association with bone-anchored limb prostheses is shown to have a 10-year cumulative risk of 20% [97]. During the 10-year follow-up, 9% of the implants were extracted due to osteomyelitis. The main pathogens involved were *S. aureus* and coagulase-negative staphylococci (CoNS). The way they are related to the presence of a specific biomaterial surface morphology is largely unknown. *In vitro* studies report contrasting results regarding the relationship between bacterial adhesion/biofilm formation and rough versus smooth surfaces [101, 102]. Nevertheless, percutaneous, porous, metal implants resulted in a lower risk of infection when compared with smooth metal implants in rabbits infected with *S. aureus* [103], indicating the importance of a structural tissue barrier to reduce the migration of skin microbes to the underlying tissues.

It is possible that the non-integrated percutaneous situation permits continuous colonisation and probably also an ongoing selection mechanism. This could result in a specific microbiota, which combines the flora of the skin with

bacteria that thrive in the specific skin-implant niche. In addition to an attempt to identify the skin flora in relation to infection around the abutment of BAHS using a standard culture [104], little is known about the microbiota on the abutment or its interaction with the commensal skin flora. The way, in detail, the skin microflora changes in the presence of a percutaneous implant is largely unknown. Specifically, knowledge of the normal commensal bacterial flora associated with BAHS and whether the pathogenicity can be affected by the environment or the characteristics of the biomaterial is limited. The opportunistic pathogen, *S. epidermidis* is able to evade the host immune response and antibiotics by invading the surrounding host tissue cells [91, 105, 106]. These studies indicate that the colonisation of the peri-implant tissue may be an important route for biomaterial-associated infection. Therefore, defining the microbiota not only on the implant surface but also in the surrounding skin could provide us with a clearer and more complete picture in order to comprehend this problem by specific antimicrobial strategies like biomaterial modifications or targeted treatments.

1.7.4 Stability

The effects of mechanical stress at the tissue-implant interface have been recognised in the biomaterial literature. A discrepancy in the elastic modulus between the abutment and the soft tissue produces interfacial strain concentrations, leading to micro-trauma and cell activation that may lead to a constant inflammatory state in the soft tissue close to the implant [107-109]. Various concepts have been proposed for distributing the stresses, particularly for implants only anchored in soft tissue where various designs for a stabilising flange have been proposed [3, 110]. Animal experiments have demonstrated an association between flange flexibility and fibrosis development as well as the amount of FBGCs adjacent to the flange [111].

For percutaneous applications such as BAHS, limb prostheses and craniofacial screws, the stability is solved by having the device anchored in bone via osseointegration. Even then, however, inflammation and infection are still a problem [97]. There is a discrepancy in the elastic modulus between a rigid abutment penetrating the skin and the surrounding tissue, resulting in stresses at the interface between the implant and tissue [112]. It has therefore been proposed that biomaterial design should focus on reducing unfavourable mechanical stresses around the implant by adapting the elastic modulus to the elasticity of the tissues, although this is rarely a possibility [107]. The transmission of skin movement around a rigid percutaneous post that generates strains or pressure at the interface is a relatively unexplored and underappreciated aspect of the tissue response. It is therefore of great interest to explore whether surgical procedures to minimise relative movement around

BAHS abutments (e.g. tissue reduction) generate a different tissue response than soft-tissue preservation techniques.

1.7.5 Surgical technique

Surgical technique is quoted anecdotally as a means of lowering the incidence of pin infection, for example. The goal is to prevent injury to the bone and soft tissues and the subsequent bacterial colonisation of necrotic tissue. Intra-operative precautions such as protecting soft tissues with drill sleeves, using sharp drill bits, avoiding thermal necrosis when using power drills and preventing ischaemic necrosis of the skin without excessive skin tension are all measures thought to reduce the risk of infections [113]. Although these techniques have not been studied formally, they represent good practice. Taking measures to reduce the thermal and mechanical damage to bone during drilling is equally important, as these factors have been linked to implant loss [114].

1.7.6 Maintenance and longevity

For percutaneous implants, both the maintenance and the treatment regimen in the case of adverse tissue responses vary, depending on device, country and individual clinics. Despite numerous studies in the area of pin-site infections to generate evidence-based treatment recommendations, substantial controversy exists with regard to the optimal protocol [113].

1.7.7 Outcome

How is successful treatment using percutaneous access monitored and evaluated and what is the definition of successful treatment? Outcome measures are often clinician-centred, measuring aspects that are deemed important primarily to healthcare professionals. It follows that patient-centred outcomes should be prioritised when both assessing individual practice and reporting the results of clinical trials.

A grading system to standardise the reporting of soft-tissue reactions BAHS was introduced by Holgers et al. and has since been used [115]. Moreover, in the field of limb prostheses and external fixators, similar systems exist [113, 116]. The Holgers scale is a macroscopic assessment of the skin surrounding the percutaneous post. It is scored on a five-point scale with 0, no signs of soft tissue reaction; 1, mild inflammation with slight redness; 2, moderate inflammation with redness and slightly moist skin; 3, redness, moist skin and granulation tissue; 4, an infection for which the removal of the implant and/or abutment is needed. Holgers grade 2 or higher is regarded as an adverse soft-tissue reaction in need of (local) treatment. This scale has never been validated

and the correlation between the scale and biological markers is unknown. While of interest to clinicians to guide the treatment required, the need for the further treatment of infected skin requiring more hospital visits and time off work or revision surgery is of greater importance to patients. Further, the scale is not a patient-centred outcome, e.g. it does not take account of parameters such as pain or aesthetics.

A standardised, well-defined set of outcome measures would improve reporting and provide a better opportunity for comparisons between studies and possibly even between treatment areas. The recently established AuroNet aims to aid in the creation of a standardised outcome set of this kind [117]. Similar efforts have been successfully implemented within dental implant treatment [118] and rheumatology [119]. In an attempt to include more aspects relevant to implant treatment, a new scale with outcome measurements in three domains, inflammation, pain and sensibility, has been proposed in the field of hearing implant treatment [120].

1.8 Stoma continence port

Approximately 700,000 people in Europe are living with an ostomy and there is an annual rate of 170,000 new operations. Stomas are needed to treat a multitude of diseases and conditions, such as colorectal or bladder cancer, inflammatory bowel diseases, diverticulitis, bladder malformation and urinal or faecal incontinence. Roughly half of all ostomies are permanent and they are equally divided between ileostomies and colostomies. When created properly, an ileostomy, colostomy or urostomy can dramatically improve a patient's quality of life. Patients with a well-functioning stoma can expect to lead a normal life with very few lifestyle restrictions. In contrast, when a patient develops complications related to his/her stoma, the impact on physical and psychosocial health can be irreparable. The associated morbidity and overall function of a stoma are dependent upon the indication for the stoma, whether it was created electively or in an emergency, and patient factors. Despite advancements in the creation and care of stomas, significant morbidity is associated with stoma creation, conveying high rates of both early and late-term complications [121-123]. In a recent evaluation of 207 patients, one or more complications occurred in 35% of the patients (27% ostomy complications, 11% peristomal skin complications) [124]. Early complications include stomal ischemia/necrosis, retraction, mucocutaneous separation and parastomal abscess. Late complications include parastomal hernia, prolapse, retraction, and varices [122]. Peristomal skin complication and constipations are other common complications. The literature reports a rate of stoma-related complications ranging from 20% to 70% [125].

Attempts have been made to overcome the problems of a conventional stoma by creating a continent ileostomy or urostomy, complex constructions often associated with a considerable morbidity and failure rate [126-128]. The continent ileostomy and urostomy, or Kock pouch, where an ileal reservoir with a continence-preserving nipple valve is constructed, offers good continence in most cases [129, 130] (Figure 2). However, the technique is complex and associated with a high failure rate and frequent need for reoperations to restore continence, mainly due to a malfunctioning nipple valve [131]. Several attempts to stabilise the nipple valve using external removable devices, implants or stapling have been unsatisfactory and it is therefore still the Achilles heel of the constructions [126, 131, 132].

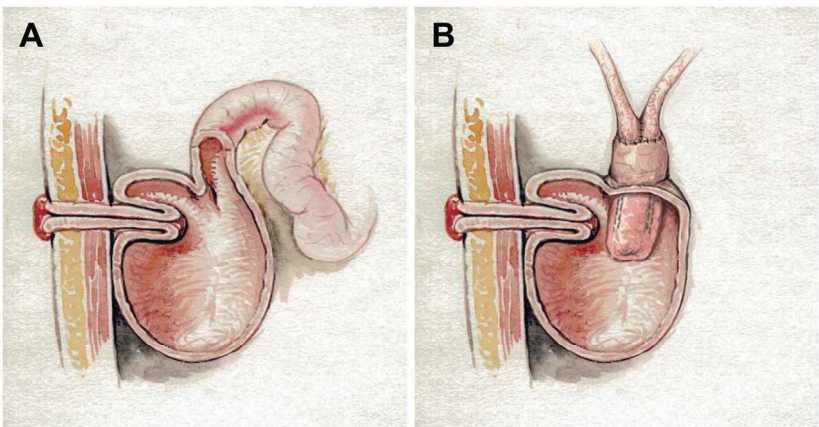

Figure 2 *Continent ileostomy (A) and continent urostomy (B). Images reproduced by kind permission of Leif Hultén.*

With reference to the background of osseointegrated permanent percutaneous implants, it is of interest to explore the opportunity to create a continent uro- and ileostomy, where the problematic nipple valve is replaced by a percutaneous implant. Experience with soft-tissue-anchored percutaneous implants used as ports for intracorporeal access for various applications in man is, however, limited, often with infection as a common finding [79, 133-135]. The anatomical position of a port of this kind and its anchorage in the soft tissue have been proven to be important factors for successful results [3, 85]. It is therefore considered of special interest to develop a design favouring the ingrowth of connective tissue to exert optimal support.

A prerequisite for a continent and leak proof stoma would be a safe, stable junction between the bowel wall and the implant, subsequently allowing a continent, leak-proof stoma. Knowledge of the biological prerequisites for connection to visceral organs is, however limited. The visceral serosa covering the organs in the abdomen including the mesentery and bowel consists of an

outer monolayer of mesothelial cells resting on a thin basement membrane connected to connective tissue. The main functions of the mesothelium are to provide a protective barrier and an adhesion-preventing surface for the free movement of apposing organs in the abdominal cavity. However, findings suggest that, with appropriate stimulation, it is able to transdifferentiate from epithelial-like characteristics to a more fibroblast-like phenotype [136]. It would therefore be of interest to explore the opportunity to anchor and integrate the bowel wall to a percutaneous implant equipped with an internal mesh structure.

1.9 Bone-anchored hearing system

A person who is not able to hear as well as someone with normal hearing – hearing thresholds of 20 dB or better in both ears – is said to have hearing loss. According to the World Health Organisation (WHO) more than 5% of the world's population – 360 million people – have a disabling hearing loss (328 million adults and 32 million children) [137]. A hearing loss affects an individual's ability to hear and communicate with others. In addition to these functional implications it might also have social and emotional consequences, as exclusion from communication will impact everyday life and can lead to social withdrawal and feelings of loneliness [138]. For children, the consequence is often delayed speech understanding and learning [139]. WHO recently estimated that the annual cost of unaddressed hearing loss was in the range of $750 billion globally [140]. The hearing loss can be unilateral or bilateral and may be mild, moderate, severe, or profound. The causes of hearing loss and deafness can be divided into congenital (present at or acquired soon after birth, e.g. microtia) and acquired (e.g. chronic otitis media, sudden deafness) causes.

1.9.1 The bone-anchored hearing system

The hearing loss categorisation relevant for treatment with a bone-anchored hearing system (BAHS) can be described as

- conductive – there is a problem conducting sound waves anywhere along the route through the outer ear, tympanic membrane (eardrum), or middle ear (ossicles)
- sensorineural – the root cause lies in the inner ear or sensory organ (cochlea and associated structures) or the vestibulocochlear nerve (cranial nerve VIII) or neural part
- mixed – a combination of conductive and sensorineural
- A single sided deaf (SSD) patient has normal or close to normal hearing in one ear and profoundly impaired hearing in the other ear.

Patients with these types of hearing loss, who can still benefit from the amplification of sound, may be candidates for a bone-anchored solution. The sound processor sends sound directly to the cochlea via bone conduction. The sound signal bypasses the conductive element of the hearing loss (the air-to-bone gap) and less amplification is therefore required compared with conventional hearing aids. Besides the types of hearing loss described above, patients with other medical indications, such as skin allergy, external otitis or ear canal stenosis, may also be candidates for a bone-anchored hearing system. The audiological features and outcomes with this bone-conducting implant treatment are not further discussed in this thesis.

The BAHS system was developed and put into clinical practice in the late 1970s [19]. Since then, the implant system, sound processor, surgical technique and indication have evolved and expanded. Currently, two major skin-penetrating systems exist and are in clinical use (Cochlear Nordic AB, Mölnlycke, Sweden, and Oticon Medical AB, Askim, Sweden).

A percutaneous bone-anchored hearing system consists of a bone conducting sound processor connected to a skin-penetrating abutment that is mounted on an implant (Figure 3A). The implant is a screw-shaped, threaded titanium implant with a machined surface which is inserted in the temporal bone behind the ear (Figure 3B). After being osseointegrated, it provides permanent anchorage as a means of attaching the sound processor and acts as a path of transmission for the vibrations that are generated. The abutment is a replaceable percutaneous connection between the implant and the external sound processor. The function of the abutment is to act as a point of attachment for the sound processor coupling and path of vibration transmission. The abutment is made of commercially pure (c.p.) Ti Grade 4, has a machined surface and is available in different lengths, for patients with different skin thicknesses (Figure 3C).

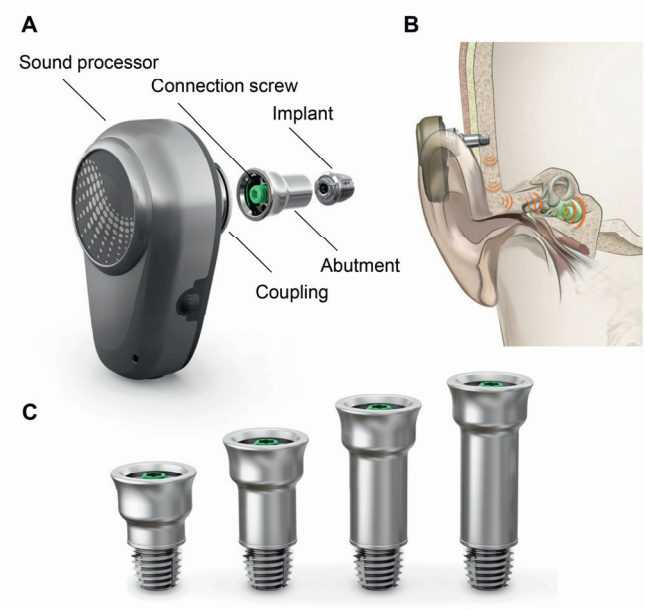

Figure 3. *Overview of a bone-anchored hearing system comprising a titanium implant, abutment and connection screw securing the abutment to the implant. The sound processor is attached to the abutments proximal end via the coupling (A). The implant is installed in the temporal bone behind the ear. Vibrations generated by the sound processor are transmitted directly through the skull bone to the cochlea as bone conduction sound (B). Detailed view of the different abutments, premounted on the implants (C). Images reproduced by kind permission of Oticon Medical AB ©. 33*

1.9.2 Implant design

Bone formation and remodelling around an implant are prerequisites to support loads during function. Titanium implants have been used in implant dentistry since the 1960s and are widely regarded as routine treatment in many treatment areas such as dental and maxillofacial applications. Originally, the implants for dental applications, as well as for BAHS, were machined with a smooth surface and this is therefore the best documented implant surface with over 40 years of clinical results [141]. Several clinical studies have been published in relation to the original osseointegration concept and a meta-analysis of 73 prospective studies of dental implants comprising 16,935 implants showed an overall success rate of 92.8% [27]. A second generation of implant modifications then emerged with, for example, blasted and acid-etched surfaces, in an attempt to accelerate and improve implant osseointegration [24].

An intentional surface modification of a biomedical implant material can be performed to promote biological reactions at the interface. In bone, these

surface modifications are designed to influence the biological events that lead to bone formation. Important key features of implant surface modifications are, first, that important bulk properties are retained and, second, that the positive biological reactions that are elicited persist, leading to maintained long-term integration and function. Most surface modifications of clinically available oral implants employ techniques that increase the roughness of the surface, compared with the machined titanium surface, resulting in surface irregularities with different forms, shapes and sizes [24]. Most of these roughened surfaces are produced by blasting, abrading and coating methods using different material particles and/or by chemical methods. In a review of oral implants, moderately rough implants were considered to have the potential benefit of a "stronger bone response and tendency towards better clinical results than turned implants."[23].

1.9.3 Surgical approaches

The procedure for placing a percutaneous implant for a bone-anchored hearing system was first described by Tjellström and co-workers in 1981 [19] (Figure 4A). In this original procedure, no soft-tissue reduction (or skin graft) was performed. A few patients complained of minor skin irritation during the first weeks after the surgical procedure. This problem was noted among those patients who had a thick subcutaneous layer and a skin rich in sebaceous glands, but no adverse skin reactions or removal of abutments was noted [19]. In subsequent papers, the long-term follow-up of the same patients was published [142]. Here, it is noted that the soft tissue had to be trimmed to avoid tissue movement around the abutment in a few cases. After the surgical reduction of the subcutaneous layers the problems disappeared. There were, however, no scientific investigations during this period comparing surgery with skin thinning with surgery without skin thinning using longer abutments. Until 1999 the shape of the abutment also included a counter-torque grip with sharp edges about halfway up on the outside of the abutment, so, if the skin was thick it met the sharp edge irritating the skin (Figure 4B).

Following the initial findings with skin irritation for patients with thick skin, the surgical procedure was modified to include a free circular skin flap and thinning of the skin surrounding the abutment for all patients [143] (Figure 5). This step in the process originated from the assumption that minimising the relative movement between the surrounding skin and the percutaneous post was necessary [144, 145]. This could be achieved by reducing the subdermal tissue leaving only the epidermis, dermis and the periosteum in contact with the abutment. The aim was to provide a thin hairless skin site that could attach to the bony layer.

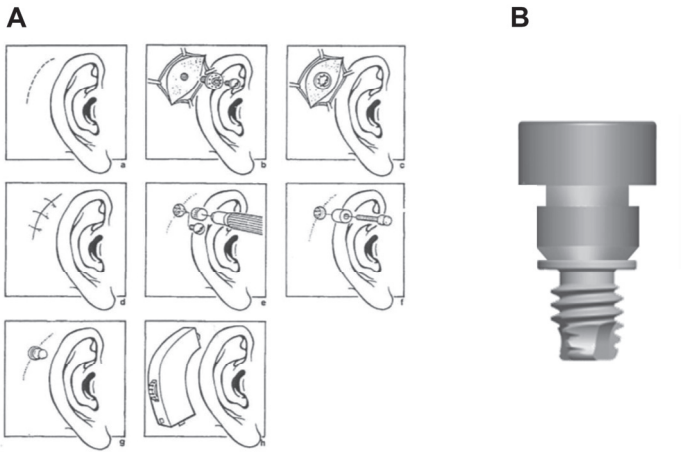

Figure 4 *(A) Original surgical procedure presented by Tjellström et. al. (1981). Am J Otol 2(4): 304-310. [19]. Reproduced with permission from Wolters Kluwer Health, Inc. http://journals.lww.com/otology-neurotology. (B) BAHA® abutment design 1978-1998 with a sharp edge facing the skin.*

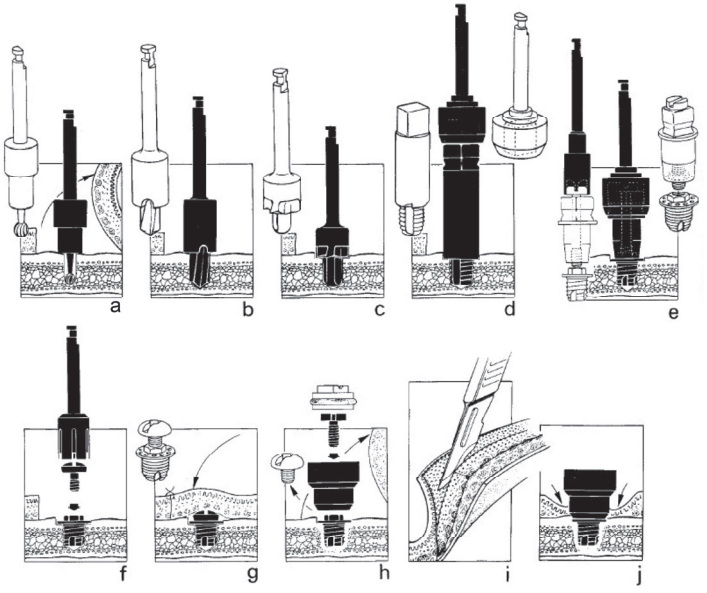

Figure 5 *Schematic illustration of the classical two-stage surgical technique for installing BAHS, from drilling to the insertion of the fixture (a-f). After closing the skin incision, it is left to heal for three to four months (g). In a second surgical procedure (h-j), the skin is again opened and an abutment is attached. In the final stage, subcutaneous skin thinning is performed. Image from Tjellström, A., et al. (1983). Acta Otolaryngol 95(5-6):568-75 [143]. Reproduced with permission from Taylor & Francis Group, www.tandfonline.com.*

Over the years, the surgical procedure was adjusted to include several different approaches, such as different skin graft techniques and various skin flap techniques with or without the use of a dermatome [146]. Independent of the technique that was used, the skin surrounding the abutment was thinned. Subsequently, the group in Nijmegen, The Netherlands, developed a linear incision technique with tissue reduction. The short-term outcome was promising and was confirmed in a long-term follow-up [147, 148]. In an assessment of the outcome using these various tissue reduction techniques, lower complication rates were shown with the less invasive approach [149].

It has been argued that the appropriate reduction of the soft tissue around the skin-penetrating abutment is vital for successful treatment, although not specifically investigated, independent of the exact technique that is used. However, reducing the skin thickness is associated with its own set of adverse outcomes, including infection, numbness, hair loss and scarring [99, 150]. Potentially, the immune host defence is weakened, as much of the soft tissue is removed and the microvascularisation is compromised [151-153]. Longer abutments and improved abutment geometries have, however, created an opportunity to perform the surgery without any skin reduction. This new technique, which preserves the surrounding soft tissue in its natural condition as much as possible, has now been practised at many hospitals for several years. In 2009, the implantation of percutaneous bone-anchored hearing implants without the traditional skin thinning was discussed for the first time at a scientific conference. The first clinical results were published by Hultcrantz in 2011 [154]. Since then, surgery with tissue preservation has rapidly gained in popularity among surgeons. The early results were promising and, recently, studies with a longer follow-up have confirmed these results [155-157].

1.9.4 Outcome and complications

To investigate the outcome after BAHS surgery a literature search was performed. Pubmed was searched using the search term "((osseointegrated hearing aid) OR (bone conduction implant) OR (bone anchored hearing) OR BAHA OR BAHS OR BAHI NOT (BAHI[Author]) NOT (BAHI-*[Author]) NOT (BAHA[Author]) NOT (Al baha))". Publications were issued between 1 January 2012 and 30 September 2017. Any article reporting outcome and/or implant loss rate after BAHS surgery was included, irrespective of age, BAHS system or surgical approach. A total of 597 publications were found and, after applying the exclusion criteria and reviewing the full text, 87 publications were included in the review [30, 34, 90, 152, 155-237]. The reviewed publications include more than 7,500 patients and 8,500 implants. Whenever possible, consideration has been taken of double reporting; some additional non-

confirmed cases of double reporting can be suspected but not confirmed, but this should not affect the overall analysis. Most of the studies (53%) comprised 10-50 patients, 33% contained more than 50 patients and 14% fewer than 10 patients. Sixty-five percent of the studies are non-controlled. The remaining thirty-five percent are controlled, whereof 68% are studies of different surgical methods. Only two per cent of the studies are randomised controlled studies. Due to these different study designs, a meta-analysis of data was not possible.

Implant survival

Sixty publications included information on implant loss due to lack/loss of osseointegration or trauma. The calculation of a true implant survival rate is not possible due to the difference in study design, but, *without* considering study design, surgical method, patient age, follow-up time and so on, the implant survival in the reviewed publications (n=7,042 implants) was 94% (range 60-100%). In adults (n=4,409), the range is 89-100% and, in children (n=547), the range is 60-99% (studies with a mixed population excluded). Children run a higher risk of implant loss due to the lack/loss of osseointegration, as well as due to trauma. Calculated as above, children run a higher risk of implant loss due to trauma, 2% vs 0.3% in the adult subgroup (note that a reason is not always given for the implant loss and only those explicitly mentioned have been counted).

Elderly people are included in many publications but are only presented as a separate subgroup (≥ 65 years) in three publications, where the implant loss rate is equal or slightly higher for the elderly groups compared with the subgroup aged 17-64 years [166, 180, 231]. A few studies have reported higher implant loss rates for diabetic patients (14% compared with 5.1% in the healthy control group [193]) and patients with previous irradiation of the temporal bone (10% for irradiated compared with 0% for the non-irradiated group) [211]. No implant losses attributed to the early loading of the implant or incorrect surgical procedure have been reported in the reviewed publications.

In a recent retrospective case study of 550 implantation in The Netherlands, an overall survival rate of 93.8% for first implants was found [238]. Long-term survival rates of 98% and 92% after the one-year and 15-year follow-up respectively were found for 4-mm implants. Low age, male gender and second implantation were found to be significantly associated with implant loss.

Tissue reactions

Most of the reviewed studies monitor tissue status, but the data are subjective and the methods vary. For the BAHS, the macroscopic soft-tissue status is commonly assessed using the five-grade Holgers score, where a score equal to or above two is considered clinically relevant requiring intervention [115]. The

outcome can be reported across visits or across patients. The reported outcome is dependent on the number of visits (especially the number of visits in the early healing phase). The studies vary in follow-up time and number of visits. Only publications clearly stating outcome (Holgers ≥ 2) in numbers or graphs are included in the following summary (45 publications, 3,495 patients). Publications with fewer than 10 subjects and/or follow-up of less than three months have also been excluded. As has been concluded in other reviews [99, 151], the outcomes vary substantially. To summarise, 25 publications (n=1201 implants) reported a Holgers score across visits ranging from 0 to 19%. The median value of Holgers ≥ 2 was 4%. The Holgers score across patients was 0 to 32%, reported in 21 publications (n=1,812) with a median value of 15% for Holgers ≥ 2.

The incidence of adverse soft-tissue reactions appears to be higher for the paediatric population compared with adults. One investigation reported a higher incidence of Holgers ≥ 2 across visits for the paediatric population compared with adults (7.8% vs. 4.3%) [180]. Four publications [30, 196, 202, 205] include data exclusively relating to children (n=169). Here, the incidence of Holgers ≥ 2 across visits and per patient ranged between 11-19% and 9-32% respectively. Patients with a diverse anamnesis, gender and ethnicity are often included in studies but are rarely accounted for separately. However, a significantly higher rate of major skin complications has been reported in the African-American subgroup (63%, n=8) compared with the Caucasian (11 %, n=38), and the Hispanic (20%, n=10) patients [237]. A predisposition for adverse skin problems in BAHS has also been demonstrated for patients with darker skin and patients with a high body-mass index (BMI) [167].

A meta-analysis of 2,310 device installations provides the best overview of outcomes with the traditional approach involving tissue reduction [99]. The reported incidence of adverse skin reactions (Holgers score ≥ 2) in adults was 2.4% to 38.1%. The reported incidence of peri-implant infection was 1.0% to 50.0% and the incidence of soft-tissue overgrowth of the abutment was 9.5% to 28.6%.

Skin overgrowth and revision surgery

A thickening of the skin around the abutment can cause difficulty attaching the sound processor and the risk of feed-back problems increases. Skin overgrowth may be reported as a complication, but it could also be included in the “revision surgery” outcome. It is therefore difficult to determine the level of this outcome. In recent reviews of outcome after installation of BAHS using soft tissue reduction techniques [99], the incidence of soft-tissue overgrowth was 10-29%, whereas, when tissue preservation techniques were used, the

incidence was estimated at up to 3% [151]. This suggests that, historically, skin overgrowth was more common due to the combination of skin thinning and short abutments. With the present availability of longer abutments, growth of the skin is mitigated by changing to a longer abutment. In severe cases, however, revision surgery or the discontinuation of use may be needed. Similarly, in this review, studies comparing tissue-preservation and tissue-reduction surgical techniques also demonstrated a decreased incidence of overgrowth when tissue preservation was used [179, 222, 231].

Numbness

Numbness, or loss of sensibility, is a common complication after a surgical procedure. In most cases, the numbness is temporary. The estimation of this outcome is subjective and highly patient dependent. In the literature, the degree of numbness is mostly reported as a dichotomous outcome (present; yes or no), reported by the patient, the surgeon or the nurse. In some cases, a ten-grade visual analogue scale (VAS) has been used, often modified to degrees of numbness; extensive (VAS 8-10), moderate (VAS 4-7), limited (VAS 1-3), none (VAS 0). Before 2000, this complication was rarely acknowledged and reported in the literature, probably as it was an inherent and unfortunate consequence of the extensive tissue reduction. Consequently, in the review of outcomes after using a tissue-reduction approach, numbness is not mentioned [99]. With the emergence of a more delicate approach with the linear incision and later tissue preservation, improvements could be seen.

Several studies report a better outcome in terms of numbness when tissue preservation is used compared with groups in which tissue reduction was performed [173, 190]. For tissue preservation surgery, the numbness also appears to resolve within one year [156, 157, 202].

Pain

Pain is a very important outcome that might hide other underlying causes. The use of the sound processor might also reduce secondary to persistent pain. As with numbness, pain is subjective and patient dependent. The degree of pain is reported as a dichotomous outcome (present; yes or no) or on a VAS scale. Chronic pain has been reported for 3-4% of the patients [171, 202] and this sometimes leads to the removal of the implant [224]. A review of the literature reveals that limited post-operative pain is reported for 2-9% of the visits up to one year [156, 157, 190, 200]. A comparison between tissue reduction (dermatome) and tissue preservation techniques demonstrated that 7% of the patients in the tissue-reduction group had limited pain at one year, while none in the tissue-preservation group did [190].

2 AIMS

The main objective of this thesis was to investigate factors influencing the outcome, in terms of tissue integration and clinical results, following the installation of a percutaneous implant system. The key factors that were evaluated were device design, surgical approach, tissue integration, infection and quantification of outcome measurements.

The specific aims of the papers included in this thesis were as follows.

- To investigate whether the integration of a titanium implant can be achieved in the porcine small intestine *in vivo* [Paper I]

- To evaluate clinically and morphologically a soft-tissue-anchored percutaneous titanium port used as a mechanical continence-preserving valve in ileo- and urostomies in dogs [Paper II]

- To determine (i) the influence of the laser modification of machined implants on implant stability, bone composition and ultrastructure, and (ii) the relationship between biomechanical (resonance frequency analysis and removal torque) and histomorphometric outcome measurements in rabbit bone [Paper III]

- To compare two drill systems with respect to cutting performance (drill force and drill torque), generation of heat and distortion of the bone during drilling *ex vivo*. Further, the study aimed to evaluate the role of specific factors (drilling procedure and effectiveness of irrigation) with respect to the degree of heat generation in the respective drilling system [Paper IV]

- The aims of this prospective clinical study were: (i) to compare the clinical outcome and microbiological profiles between machined and polished abutments after three months of bone anchored hearing system (BAHS) implantation; (ii) to correlate the microbiological profiles to the clinical soft-tissue manifestations, and (iii) to evaluate different sampling procedures for bacterial identification and enumeration [Paper V]

- To evaluate, in a human multicentre service evaluation, the surgical procedure and short-term outcome of a novel, minimally invasive technique for installing bone-anchored hearing implants [Paper VI]

- To describe the research protocol for a multicentre, randomised, controlled study, comparing a new minimally invasive technique for installing bone-anchored hearing implants with the linear incision technique, assessing and comparing (i) the incidence of inflammation, (ii) measurements related to surgery, implant performance, soft tissue conditions and cosmetics and (iii) measurements related to quality of life, health economics and local soft-tissue responses [Paper VII]

- To compare the short-term results (three months) when using a minimally invasive technique for installing BAHS, with those of the linear incision technique, in terms of incidence of inflammation and measurements related to surgery, implant performance, soft-tissue conditions and cosmetics [Paper VIII]

3 MATERIALS, PATIENTS AND METHODS

The thesis is based on experimental *in vivo* [Paper I, II, III], bench/*ex vivo* [IV] and clinical [V, VI, VII, VIII] work (Table 2).

Table 2 *Summary of the* in vivo*,* ex vivo*/bench and human studies included in the thesis and their respective area of interest. MIPS=Minimally invasive Ponto surgery.*

Paper	Model	Application	Feature	Area of interest
I	Pig	Continence port for ileostomy	Titanium mesh	Exploratory Tissue integration Infection
II	Dog	Continence port for ileostomy and urostomy	Percutaneous titanium stoma implant with integrated mesh	Exploratory Tissue integration Infection
III	New Zealand white rabbit	Implant for BAHS	Screw shaped titanium implants Laser -modified (test) Machined (ctrl)	Tissue integration (bone)
IV	Artificial bone Cow tibia	Drill system for BAHS	MIPS (test) Conventional (ctrl) Drilling protocols (standard and deviations)	Surgical approach Heat generation Tissue damage
V	Human	Abutment for BAHS	Polished abutment (test) Machined abutment (ctrl)	Surgical approach Infection Outcome measure
VI	Human	Surgical technique for BAHS	Short term evaluation of MIPS	Surgical approach
VII, VIII	Human	Surgical technique for BAHS	Multicentre, randomised controlled study comparing outcome employing two different surgical approaches. MIPS (test) Linear incision (ctrl)	Surgical approach Infection Outcome measure

3.1 Stoma continence port

An exploratory experimental study on pigs was carried out using different stoma port configurations [Paper I]. These were then further developed and an experimental dog model, for the evaluation of functional ileo- and urostomies with a continence-preserving stoma port, was developed [Paper II].

3.1.1 Implant designs

The implants were machined from commercially pure (c.p.) titanium (Ti) grade 2 (ISO 5832-2). The meshes were either c.p. Ti grade 1 or grade 2. In Study II, cylindrical stoma ports with an internal laser-cut mesh structure were fabricated from c.p. Ti grade 2 (Figure 6). After assembly, the implants were blasted with aluminium oxide creating a mean surface roughness, Sa, of approximately 0.9 µm determined by interferometry (WYKO NT9100 optical profilometer, Vision v4.10 software, Veeco Instruments Inc., Plainview, NY, USA). The implants were ultrasonically cleaned before autoclaving. The test implants were manufactured by OstomyCure AS (Oslo, Norway).

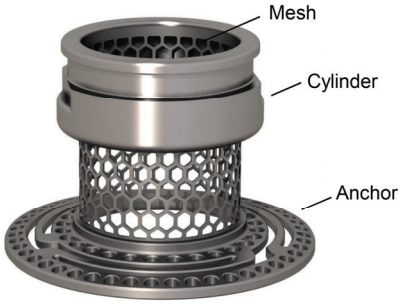

Figure 6 *Configuration of the stoma port used in Study II consisting of a skin-penetrating cylinder and a ring-shaped anchor. The perforation of the anchor ring permits the ingrowth of connective tissue. A cylindrical titanium laser-cut mesh is enclosed by the cylinder and attached to the anchor.*

3.1.2 Experimental study design – pig model [Paper I]

Female pigs (Norwegian land race) with a mean body weight of 55.5 ± 11.7 kg were used for the implantation of titanium stoma implants. After anaesthesia, an appropriate site a few centimetres below the last (distal) rib was shaved and disinfected with iodine. The insertion of the implants was approached through a midline incision. To obtain optimal information on the dependent factors influencing the tissue ingrowth of the implant and a successful outcome, the experiments were carried out systematically and in sequence, with increasingly complex implants and surgical models (Figure 7). Four different cylindrical implant configurations, with flanges for stabilisation and an internal mesh for bowel integration, were used (Figure 7).

All the implants were maintained for between one and three weeks according to plan. The implants were examined daily and the condition of local tissue was assessed in terms of inflammation, infection and other adverse events.

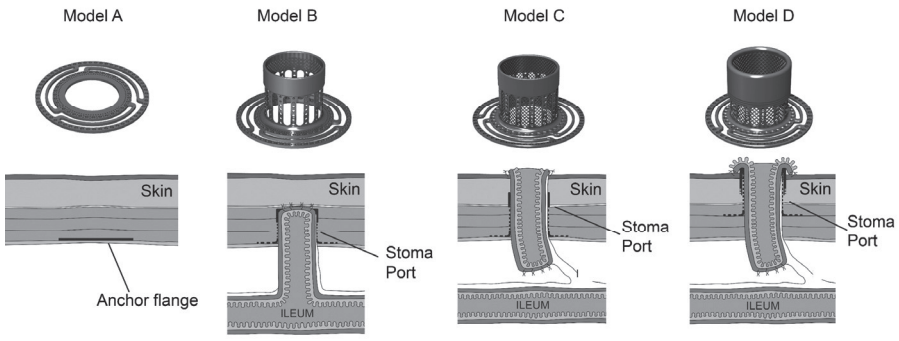

Figure 7 *Configuration of the anchor flange, stoma ports (SP), and surgical options used in Study I. Model A: Anchor flange installed peritoneally. Model B: Subcutaneous bypass ileostomy with subcutaneously positioned port. The implant was inserted with the anchor flange positioned as in Model A and the implant cylinder within the abdominal musculature. According to a Roux-en-Y bypass technique, a closed ileal segment inserted in the implant. Model C: Segment ileostomy with subcutaneously positioned port. The implant was positioned as in Model B, extending into the submucosa but not penetrating the skin. An isolated, vascularised, ileal segment is passed through the implant and fixed with a mucocutaneous suture. Model D: Percutaneous segment ileostomy and percutaneous port. The anchor flange was placed in the abdominal musculature with the implant penetrating the skin. An isolated ileal segment was passed through the implant and sewn with a mucocutaneous suture.*

3.1.3 Experimental study design – dog model [Paper II]

In purpose-bred, female, Labrador dogs (25-34 kg), the stoma port was inserted in the abdominal wall. An isolated ileal reservoir was constructed and its exit conduit was brought through the stoma port aperture, after which the skin incision was closed (Figure 8A). A catheter was introduced into the reservoir and fixed in place with a suture enabling the continuous drainage of the reservoir and flushing with saline minimum twice daily. Via the catheter, the graded distension of the reservoir was performed in order to increase its volume to about 60 ml.

In a second surgical procedure (median 4.2 weeks, range 3-10 weeks after the first stage), the small bowel (n=4) or ureter (n=3) was subsequently anastomosed to the reservoir creating a functioning ileo- and urostomy respectively (Figure 8B). For the urostomy group, the reservoir volume was then further enlarged by gradually increasing the period with a lid attached to the implant (from one hour to five hours). The implants were examined daily and the condition of the operation field and local tissue was assessed. The degree of inflammation and other adverse events was noted. The implants were maintained between three and 28 weeks after the first procedure.

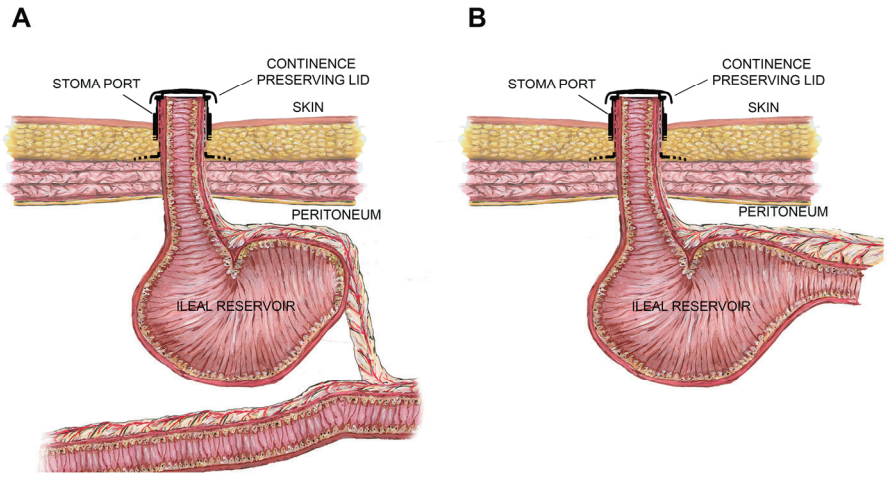

Figure 8 *Schematics showing the stoma model with the titanium stoma port used in Study II. The ports are installed in the abdominal wall with the anchor flange located in the abdominal musculature and the cylinder penetrating skin. The ileal outlet from a valve less reservoir* ad modum *Kock is passed through the port (A). At a later stage, either the ileum or a ureter is anastomosed to the base of the reservoir (B).*

3.1.4 Macroscopic and histological evaluation

At termination, a detailed macroscopic examination of the implant and surrounding tissue, as well as the ileal segment and reservoir was performed. The macroscopic appearance was observed and documented by photography. Any abnormalities or adverse macroscopic reactions were noted. The implants were then excised *en bloc,* together with surrounding tissue and the intestinal segment within the port and immersed in 4% neutral-buffered formalin. The specimens were dehydrated in increasing grades of ethanol and subsequently infiltrated and polymerized in LR White Resin (London Resin Company Ltd, Berkshire, UK). After embedding and polymerisation, the samples were cut using the Exakt cutting-grinding equipment [239]. The approximately 50µm thick ground sections of the implant and tissue were stained with either Richardson's or a modified Van Gieson stain. A qualitative light-microscopic histological examination was performed to evaluate the morphological appearance. Interest focused on the morphology of the abdominal wall and bowel in relation to the stoma port and its mesh structure. The adaptation of the tissue was determined in terms of integration, the presence and degree of inflammatory response, the degree of vascularisation, epidermal downgrowth and bacterial presence.

3.2 Bone-anchored hearing system

3.2.1 Surgical techniques [Paper V, VI, VII, VIII]

In Studies V, VI, VII and VIII, a new, minimally invasive surgical technique, the MIPS technique (Minimally Invasive Ponto Surgery), for installing BAHS was evaluated. For Studies VII and VIII, the conventional standard surgical technique, the linear incision with tissue preservation, was used as a comparison [154]. Both techniques are described below.

3.2.1.1 Linear incision with tissue preservation

This technique was first described in the literature by Hultcrantz in 2011 [154]. In contrast to previously used techniques, the skin surrounding the abutment is not thinned. The steps are described in Figure 9. A 2-4 cm long linear incision down to the periosteum is made (Figure 9A). The incision is opened up using a self-retaining retractor and the periosteum around the surgical site is removed (Figure 9B). Guide drilling is performed down to 3 mm (Figure 9C). If the bone thickness is sufficient, the spacer is removed to prepare for a 4-mm implant (Figure 9D). The hole is widened with the countersink (Figure 9E) and the implant is installed (Figure 9F). A hole in the skin over the abutment is made using a Ø 5 mm biopsy punch (Figure 9G). The skin is eased over the abutment and the incision is closed (Figure 9H). Finally, a healing cap is snapped onto the abutment. Ointment-soaked ribbon gauze is wrapped around the abutment (Figure 9I).

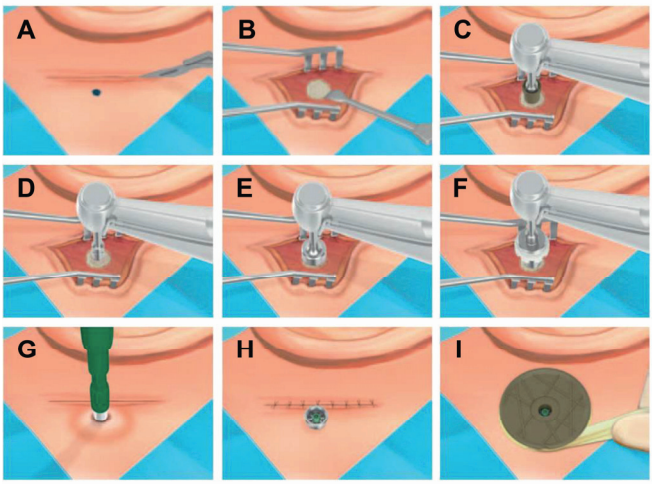

Figure 9 *Description of the linear incision technique using for installing BAHS using the conventional drill system and protocol. Images reproduced by kind permission of Oticon Medical AB ©.*

3.2.1.2 Minimally invasive Ponto surgery (MIPS)

The basic steps of the MIPS technique are the same as for any other surgery using BAHS. However, MIPS is only designed for single-stage surgery. The implant position is chosen in the same way as in any bone-anchored implant surgery and at the chosen site, an incision is made using a 5-mm biopsy punch (Figure 10A). A raspatorium is used to ensure that all the soft tissue and periosteum are removed around the surgical site (Figure 10B). The cannula is then inserted (Figure 10C). Guide drilling is performed through the cannula with the guide drill (Figure 10D). The guide drill has a spacer that is removed if the bone thickness allows a 4-mm long implant (Figure 10E). The hole is then widened with the widening drill (Figure 10F). The cannula is removed and the implant installation is performed through the circular incision (Figure 10G). Finally, a soft healing cap is attached to the abutment and a suitable dressing is applied (Figure 10H).

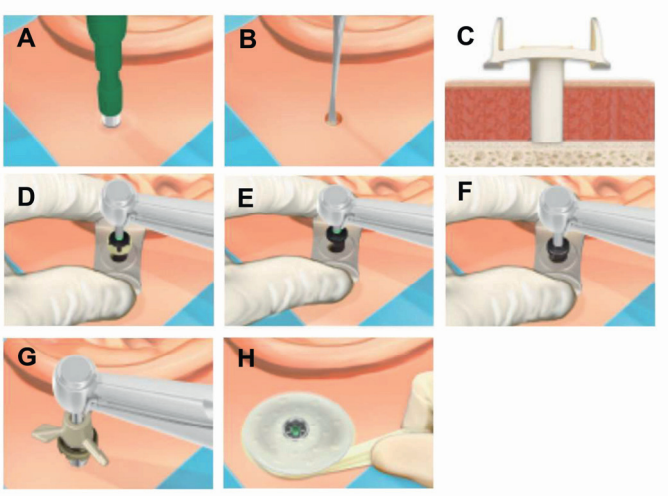

Figure 10 *Description of the minimally invasive, flap-less technique using the MIPS drill system and protocol for installing BAHS.*
Images reproduced by kind permission of Oticon Medical AB ©.

3.2.2 Drill system [Paper IV]

The conventional drill system (Ponto) consists of the initial preparation of the bone with a round burr and subsequent countersink (Figure 11A). In MIPS, the drill system consists of a guide drill for initial preparation and subsequent enlargement of the osteotomy with a widening drill, both having a twist drill design (Figure 11B). The shape of the osteotomies after each drill step for the two drill systems are shown in Figure 12. All the drills were manufactured from stainless steel and, in addition, the MIPS drills were coated with diamond like carbon. All the drills and instruments were provided by the manufacturer (Oticon Medical AB, Askim, Sweden).

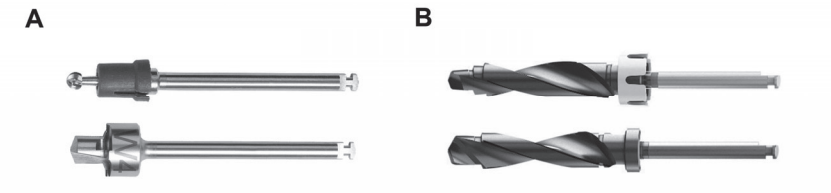

Figure 11 *(A) The conventional drill system used for the of the linear incision technique used for installing BAHS, Top: guide drill with removable spacer. Bottom: countersink, 4 mm. (B) The MIPS system for the minimally invasive, flapless technique. Top: guide drill with removable spacer. Bottom: widening drill, 4 mm. Images reproduced by kind permission of Oticon Medical AB ©.*

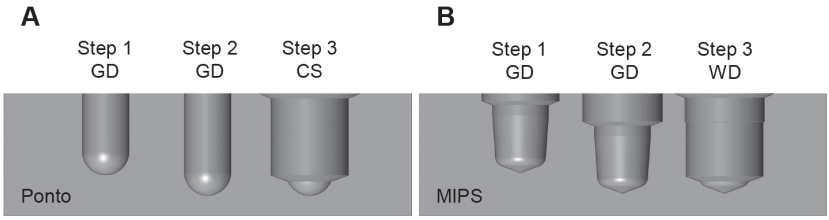

Figure 12 *Shape of the osteotomies during sequential drilling steps for the Ponto (A) and MIPS (B) systems. A 4 mm deep hole is generated with the guide drill (GD) (Step 1), thereafter deepened an additional millimetre to make it deep enough for a 4 mm long implant (Step 2). Finally, the osteotomy is widened with the countersink drill (CS) or widening drill (WD) for the Ponto and MIPS systems respectively (Step 3).*

3.2.3 Modified implant [Paper III]

In Study III, screw-shaped implants (diameter 3.75 mm, length 5 mm) were machined from c.p. Ti grade 4. Selective laser ablation with an Nd:YAG laser was employed to produce site-specific surface modification confined to the thread valley, reaching approximately 30% of the thread height on each flank, leaving most of the implant un-modified. Un-modified implants with a machined surface were used as controls. The implants were cleaned in Extran MA01® (Merck Millipore, Darmstadt, Germany) prior to sterile packaging and subsequent autoclaving.

3.2.4 Modified abutments [Paper V]

In Study V, abutments (c.p. Ti grade 4) were either left untreated with a machined surface (control) or electropolished (test) using an ElpoLux TI electrolyte (ElpoChem AG, Volketswil, Switzerland). The abutments were ultrasonically cleaned stepwise in liquid detergent, deionised water and ethanol and dried in filtered air. All abutments and implants were sterilised by beta-irradiation in a plastic blister.

3.2.5 BAHS components

In the clinical studies (Studies V, VI, VII, VIII), the BAHS system and instrumentations were supplied by Oticon Medical AB. The devices used in the studies are listed in Table 3 below:

Table 3. *Articles and article number of the implants and instruments used in the drill evaluation and clinical studies.*

	Article	**Product number**
Implants and abutments	Ponto Wide implant, 4 mm, with abutment, 6, 9, 12 and 14 mm Ponto BHX implant, 4 mm, with abutment, 6, 9, 12 and 14 mm	M51136, M51137, M51138, M52065, M52168, M52169, M52170, M52171
Implants and polished abutments	Ponto Wide implant, 4 mm, with polished abutment 9 and 12 mm	M52151, M52152
Ponto drill system	Guide drill Countersink 4 mm	M50287, M51122
MIPS system	MIPS surgery kit, 4 mm (including guide drill and widening drill 4 mm)	M52207

3.3 Material characterisation

For the characterisation of the surface morphology of the implants [Paper III] and the polished and machined abutments [Paper V], SEM was used for qualitative assessment, the surface element composition was determined using AES and the topographic parameters were determined by interference microscopy. In addition, the wettability of the abutment surface was determined by water contact angle measurements. The implants and instruments used in the clinical studies evaluating the MIPS surgical technique [Paper VI, VII, VIII] were available commercially (Oticon Medical AB) and surface characterisation was not performed.

3.3.1 Scanning electron microscopy

In a scanning electron microscope (SEM), a focused beam of high-energy electrons is used to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including morphology, chemical composition, and the crystalline structure and orientation of materials making up the sample surface. Accelerated electrons in an SEM carry significant amounts of kinetic energy and this energy is dissipated as a variety of signals produced by

electron-sample interactions when the incident electrons are decelerated in the solid sample. These signals include secondary electrons (that produce high-resolution SEM images), backscattered electrons (BSE), diffracted backscattered electrons (EBSD that are used to determine crystal structures and the orientations of minerals), photons (characteristic X-rays that are used for elemental analysis and continuum X-rays), visible light (cathodoluminescence-CL) and heat.

In this work, SEM was used to generate images qualitatively to assess the surface morphology of machined and laser-modified implants [Paper III] using a Leo Ultra 55 FEG SEM (Zeiss, Oberkochen, Germany) in the secondary electron mode operated at 5 kV accelerating voltage, using a regular secondary electron detector at low resolution and an in-lens detector at high resolution, at ×50-200,000 magnifications. The machined and polished abutments [Paper V] were analysed with the same equipment operating at 10 kV through a secondary electron detector and in-lens detector at x20-100,000 magnifications.

3.3.2 Profilometry

Interferometry is a non-contact, optical technique for mapping the surface topography of a sample. The method is based on the interference effects that occur when there is a difference in distance travelled by the light reflected from the sample and the light reflected by a high-precision reference mirror. A collimated light beam is split into a measurement beam, striking the sample, and a reference beam, striking the reference mirror. The reflected lights are superimposed at the beam splitter and focused onto a camera. The resulting fringe pattern is determined by the phase difference between the two beams. In this work, non-contact white light 3D interference microscopy was used to determine the surface topography of the implants and abutments.

The surface topography of the experimental implants [Paper III] was analysed by white light 3D interference microscopy (Wyko NT1100 optical profiler, Veeco Instruments Inc., Plainview, NY, USA). Two implants of each type were used and the measurements were performed on four threads per sample. The collected data were processed (plane fitting and missing data point reconstruction) (Veeco Vision 32 v3.43 software, Veeco Instruments Inc.). Filtering and the roughness data parameters were calculated (SPIP V3.0.0.9 software, Image Metrology A/S, Hørsholm, Denmark).

Surface topography measurements of the abutments [Paper V] was performed by white light 3D interference microscopy (Wyko NT9100, Veeco Instruments Inc.). Three machined abutments and four polished abutment samples were

analysed. On each sample, the topography was measured in three 310 x 235μm areas. Data were processed by extrapolating "invalid pixels" (the modulation threshold was set at 3%) and tilt plus cylinder shape correction, smoothed by a 3x3 median filter to reduce noise and the topography parameters were calculated (Veeco Vision v. 4.10, Veeco Instruments Inc.)

3.3.3 Auger electron spectroscopy

Auger electrons are produced following the ionisation of an atom by the primary electron beam and the falling back of an outer shell electron to fill an inner shell vacancy. The excess energy released by this process may be carried away by an Auger electron. This electron has characteristic energy and can be used to provide chemical information. A sample is exposed to a focused electron beam and ejects an electron from the inner shell of the atom. This vacancy is refilled by an electron from an outer shell with higher energy. The energy thus emitted is transferred to a secondary electron, an Auger electron, which is emitted and can be analysed by an electron spectrometer. The kinetic energy of the Auger electron corresponds to the type of atom and the chemical environment in which the atom was located. The resulting spectra can be used to determine the identity of the emitting atoms and some information about their environment. Here, AES has been used to determine the element composition of the implants and the oxide thickness.

Determination of the chemical composition of the implant [Paper III] and abutment [Paper V] surfaces was performed using Auger electron spectroscopy (AES) (PHI 700 Scanning Auger Microprobe, 3.0 keV, Physical Electronics Inc., Chanhassen, MN, USA). Two implants of each type (laser modified and machined) and four abutments of each type (polished and machined) were analysed at four locations on each sample. For the implants, the oxide thickness was determined by AES depth profiling on two implants of each type, where three areas with dimensions of 10 μm x10 μm were analysed on each sample.

3.3.4 Wettability

The contact angle, θ, is a quantitative measurement of the wetting of a solid by a liquid and is determined using an optical tensiometer. The contact angle is defined geometrically as the angle formed by a liquid at the three-phase boundary where a liquid, gas and solid intersect. In practice, a droplet is placed on the solid surface and the image of the droplet is recorded. The static contact angle is measured when the droplet is standing on the surface and the three-phase boundary is not moving and it is then defined by fitting a Young-Laplace equation around the droplet.

The surface wettability of 9 mm and 12 mm polished and machined abutments was determined by contact angle (θ) measurement at room temperature [Paper V]. A 3µl droplet of deionised water was dispensed on the cylindrical part of the abutment using a syringe and the static contact angle was determined (Theta Lite optical tensiometer, One Attension software v2.6, Biolin Scientific, Gothenburg, Sweden).

3.4 Experimental study designs

Laser-modified and machined, screw-shaped implants were installed in the tibiae of New Zealand rabbits to evaluate of the osseointegration [Paper III]. Evaluations of the drill system in terms of heat generation and drill mechanics were performed in an artificial bone model, whereas the evaluation of the distortion was performed in *ex-vivo* bovine tibia [Paper IV].

3.4.1 Osseointegration study [Paper III]

Adult female New Zealand white rabbits (Lidköpings Kaninfarm, Lidköping, Sweden; weighing 4-5 kg) received one implant of either type, laser-modified and machined, in each proximal tibial metaphysis for a healing period of eight weeks. The surgical site was shaved and cleaned using chlorhexidine digluconate (5 mg/mL in 70% ethanol; Fresenius Kabi, Uppsala, Sweden). The bone surface was exposed by the incision and blunt dissection of the underlying tissue, including the periosteum. The drill holes were prepared by a stepwise enlargement, starting with a 2-mm diameter round burr, followed by a 2-mm twist drill, 3-mm pilot drill and a 3-mm twist drill under copious irrigation with saline. The final preparation was made with a screw tap prior to installing the implants at slow speed (OsseoSet^{TM} 200 drill unit, Nobel Biocare AG, Zurich, Switzerland). The implant stability quotient (ISQ) was measured by means of resonance frequency analysis (RFA) (Osstell Mentor system, Osstell AB, Göteborg, Sweden). Two measurements were made at 90° to each other. The ISQ was measured again prior to the sacrifice of the animals after eight weeks.

The animals were euthanised by an intravenous overdose of sodium pentobarbital (60 mg/mL). A removal torque (RTQ) evaluation was made using a torque-testing machine connected to the implants via a custom fabricated adapter. The system was aligned and calibrated. The RTQ value was monitored in real time while rotating the implants at a constant angular speed of 0.2°/s.

3.4.2 Drill system evaluation [Paper IV]

Closed cell polyurethane foam (REF 1522-27, Sawbones Europe, Limhamn, Sweden), simulating hard, human bone, was used for the mechanical evaluation and the measurement of heat generation.

The MIPS drill system was evaluated with respect to heat generation and compared with the temperature generated by the conventional Ponto drill system. Five different drilling procedures, chosen to imitate different clinical situations in order of assumed increased heat generation due to deviation from the recommended standard protocol, were employed for each of the two drill systems (Table 4).

Table 4 *Description of the five different drilling procedures employed for the heat generation evaluation.*

ID	Description
DDI	Direct drilling with continuous irrigation. Drilling according to the recommended standard procedure. Direct drilling in a one-step continuous down-and-up motion during continuous irrigation and flushing of the osteotomy after removal of the drill bit from site.
DDI3	Direct drilling with idling for two seconds. Similar to DDI with the addition that each drill is left idling in the osteotomy (rotating at 2000 rpm) for approximately two seconds after reaching the full depth.
DDII	Direct drilling with impaired irrigation. Similar to DDI with the modification of impairing the irrigation (for Ponto, water is administered on to the bone bed prior to each drill step, no continuous irrigation during drilling, no flushing of osteotomy after removal of drill bit; for MIPS, the cannula is filled with water prior to each drill step, no continuous irrigation and no flushing after drilling).
DDII3	Direct drilling with idling for two seconds and impaired irrigation). Similar to DDI but with the combination of idling (DDI3) and impaired irrigation (DDII).
DD	As a final worst-case (positive control) condition, direct, continuous drilling without irrigation.

The quality and degree of bone damage at the drilling site was evaluated by drilling in *ex vivo*, bovine, compact, tibial bone. Three osteotomies were created with each of the systems during standard procedure and irrigation. Immediately after the osteotomy procedure, the blocks were fixated in formalin and subjected to histological preparation and evaluation.

3.5 Clinical study designs

In this work, two clinical investigation [Papers V, VII, VIII] and one clinical service evaluation [Paper VI] were performed (Table 5). The multicentre, service evaluation comprised 76 patients and 77 implants [Paper VI]. It focused on intra-surgical results and short term clinical outcome related to soft tissue after using the MIPS surgical technique for installing BAHS. The prospective clinical investigation, involving twelve patients, compared the clinical outcome and microbial profiles between machined and polished abutments up to one year after BAHS surgery using the MIPS technique [Paper V]. The multicentre randomised clinical investigation comprised 64 patients, each receiving BAHS, using either the MIPS surgical technique or the linear incision technique with tissue preservation (*ad modum* Hultcrantz) [154] [Papers VII, VIII].

Table 5 *Summary of the clinical studies included in this work.*

	Paper V	**Paper VI**	**Paper VII, VIII**
Study type	Single centre, prospective controlled case series clinical investigation	Multicentre, prospective service evaluation	Multicentre, open, randomised, controlled clinical investigation
Follow-up	One year	Surgery and first two visits	Two years
Interventions	Installation of BAHS using MIPS technique	Installation of BAHS using MIPS technique	Installation of BAHS
Investigational technique/device	Polished abutment (n=5)	MIPS surgical technique (n=76)	MIPS surgical technique (n=33)
Comparator(s)	Machined abutment (n=7)	N/A	Linear incision surgical technique *ad modum* Hultcrantz (n=30)
Primary objective	To compare two different topologies of abutments for BAHS in terms of bacterial colonisation, inflammatory response and skin reactions.	To evaluate the surgical procedure and short-term outcome when using MIPS for installing BAHS	To compare the incidence of inflammation between the test and control group after three months post-surgery

	Paper V	Paper VI	Paper VII, VIII
Secondary objective(s)	To compare between groups: – The microbiological profiles (bacterial counts at the abutment-skin interface) – The clinical outcome To correlate the microbiological profiles to the clinical soft tissue manifestations To evaluate different sampling procedures for bacterial identification and enumeration	Intra-operative data: – Surgery time – Deviations from instructions – Intra-operative events. The postoperative outcome from the first two follow-up visits was recorded using measurements routinely collected for BAHS, such as sensory outcome, complications, treatments and implant loss. Skin reactions were registered according to the Holgers scale	To compare performance indicators between groups at three and 24 months post-surgery: – Presence of dehiscence – Pain – Loss of sensibility – Soft tissue overgrowth – Extrusion – Cosmetic results – Surgical procedure time – Wound healing – ISQ values – To evaluate the skin position and movement of the skin around the abutment
Primary endpoint	CFU counts three months post-surgery	N/A	Incidence of inflammation between surgery and three months post-surgery
Inclusion criteria	Any adult (18 years of age or older) patient eligible for a single-stage BAHS surgery	Any adult (18 years of age or older) patient eligible for a single-stage BAHS surgery	(i) if they will undergo unilateral BAHS surgery and (ii) when they are ≥ 18 years of age.
Exclusion criteria	Exclusion criteria – Inability or unwillingness to participate in follow-up – Skin thickness of > 10 mm – Diseases known to compromise bone quality – Irradiated in the implant area Withdrawal criteria – Skin thickness of > 10 mm – Use of surgical procedure other than MIPS	According to local clinical practice	Exclusion criteria (i) History of immunosuppressive disease (ii) Use of systemic immunosuppressive medication (iii) Bilateral BAHS implant 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

3.5.1 Polished abutment for BAHS [Paper V]

Enrolled patients were allocated consecutively to the control (machined abutment) and the test (polished abutment) groups. Using the MIPS technique, patients received the Ponto wide implant (diameter 4.5 mm, length 4 mm), pre-mounted with either a machined or an electro-polished abutment of suitable length (Oticon Medical) [200, 240]. After surgery, the patients were assessed at 5-10 days, 3-12 weeks, 12 weeks, six months and 12 months (Figure 13). Samples for the identification and quantification of colonising bacteria were taken from three different compartments: on the abutment, in the peri-abutment exudate and in the soft tissue next to the abutment (Figure 13). Sampling was performed at baseline (only tissue biopsy), three months (all three compartments) and 12 months (all three compartments). Clinical outcome measures such as the Holgers score, hygiene, pain, numbness and implant stability (ISQ) were investigated on all follow-up visits.

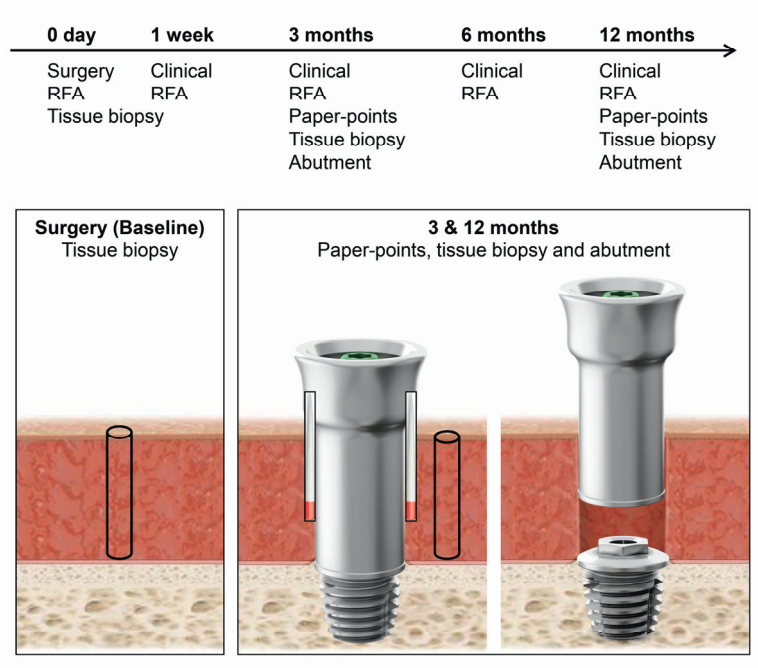

Figure 13 *Study outline and schematics of sampling tissue with a biopsy, peri-abutment fluid with paper-points and retrieval of abutment, at baseline, three and 12 months. Parallel to the microbiological sampling, clinical measurements were collected and the stability of the implant was assessed with resonance frequency analysis (RFA).*

3.5.2 MIPS service evaluation [Paper VI]

In this multicentre case-series evaluation, the surgical results and short-term outcome when using the first-generation MIPS surgical system to install BAHS were assessed. Twenty-one surgeons from 15 centres across Europe participated in the evaluation. The surgeons were experienced in installing BAHS using classical methods and were given MIPS training prior to the first surgery. Only adult patients eligible for single-stage bone-anchored surgery were included. Patient characteristics such as gender and age span (18-50; 50-75; >75 years) were collected. Intra-operative data such as implant and abutment used, skin thickness, type of anaesthesia, bone quality, surgical length, deviations from standard instructions, complications and any issues with instrumentation were recorded. Post-operatively, the patients were followed for the first two follow-up visits planned according to local practice. In addition, any unplanned visit within this time window was recorded. At any follow-up visit, all centres recorded implant survival, skin reactions according to Holgers score and any other post-operative complications or treatment needed.

3.5.3 MIPS multi-centre clinical investigation [Papers VII, VIII]

In this work, a multicentre, open, randomised, controlled investigation was designed to compare the MIPS technique with the linear incision technique with soft-tissue preservation. Sixty-four participants were included at three centres in The Netherlands. Enrolled patients were allocated consecutively to the test group (MIPS) or the control group (linear incision technique with soft-tissue preservation) in a 1:1 ratio stratified for gender (Figure 14). Patients were assessed at inclusion, surgery, standard follow-up visits (nine days, three and 12 weeks, one and two years) and extra consultations. The primary end-point was the incidence of peri-abutment inflammation (Holgers ≥ 2) between surgery and at the 12-week follow-up. Secondary outcomes included 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. Intra-operative complications, post-surgical complications, adverse events, serious adverse events and device deficiencies were also noted.

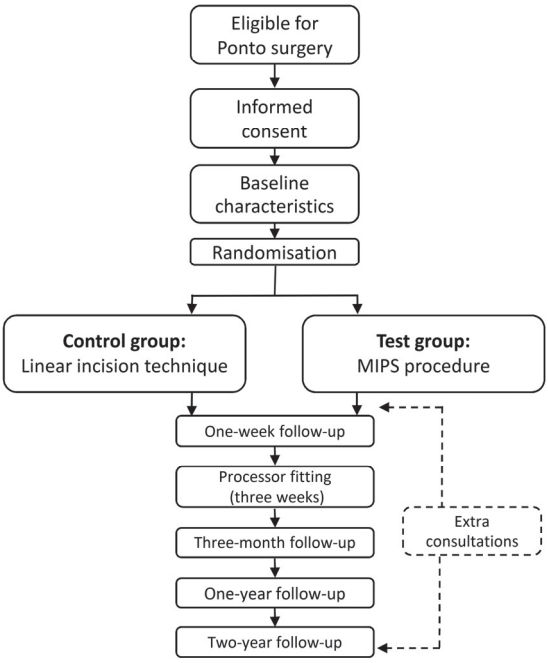

Figure 14 *Study flow chart of the multicentre, randomised, controlled investigation was designed to compare the MIPS technique with the linear incision technique with soft tissue preservation.*

3.6 Analytical techniques

3.6.1 Biomechanical techniques

The biomechanical evaluation of implants is designed to give a quantitative measurement of implant stability and anchorage to the bone. Resonance frequency analysis (RFA) is the measurement of the frequency by which the implant-bone unit vibrates. By attaching a peg to an implant or abutment and subjecting it to magnetic pulses, the vibration frequency can be measured. The implant stability quotient (ISQ) is a value on a scale from 1-100 that corresponds to the vibration frequency between 1 and 10 kHz. The ISQ value is influenced by the bone density, osteotomy configuration, implant (and abutment) design and degree of osseointegration and it is commonly used in the field of bone-anchored hearing systems to assess non-invasively the implant stability over time. In contrast, a removal torque test (RTQ) is primarily related to the bone-to-implant interfacial properties and refers to the torque needed to unscrew an implant from the bone after a specific period of healing.

In the rabbit study [Paper III], ISQ was measured prior to attaching the cover screw using the Osstell Mentor system (Osstell AB, Göteborg, Sweden). Eight weeks after implant installation, the animals were euthanised, the implants were exposed and the cover screws were exposed. ISQ was measured again prior to subjecting the implant to a removal torque test using specially designed equipment. The RTQ was measured while rotating the implants at a constant angular speed of 0.2°/s. After testing, the implants were removed *en bloc* with surrounding tissue, which was immersed in formalin for subsequent histopathological preparation. In the clinical studies [Papers V, VIII], ISQ was measured at surgery and on selected follow-up visits.

3.6.2 Mechanical techniques

In the mechanical evaluation of the two drill systems [Paper IV], artificial bone was subjected to each drill with a constant feed rate of 1mm/sec and with a constant rotational speed of 2,000 rpm while measuring thrust force and torque, using a specially designed test rig (Torque Test Rig, Asset No. 1002, Biomekaniska Laboratoriet AB, Billdal, Sweden) (n=10). For both drill systems, five drilling procedures with a feed rate of 1 mm/s were recorded for three individual sets of drills (a total of 15 measurements of drill force and drill torque for each type of drill). All drill sequences were performed without irrigation.

3.6.3 Heat generation

A thermocouple consists of two wires made of different metal materials and joined at both ends. When one end is heated, a temperature-dependent voltage is produced because of the thermoelectric effect and this voltage can be interpreted as temperature. Type K thermocouples are made of chromel and alumel and are the most common general-purpose thermocouples with a sensitivity of approximately 41 μV/°C.

To determine heat generation when drilling in artificial bone with different drill systems and drilling procedures, the temperature at the osteotomy was measured using thermocouples (type K, RS Components, Gothenburg, Sweden). These were connected to a data logger (TC-08 Data logger, PICO, Cambridgeshire, UK) allowing constant, real-time temperature readings. At the planned osteotomy sites, four canals of different depths were drilled to house the thermocouples at a distance of 0.5 mm from the calculated final periphery of the osteotomy (Figure 15). A surgical drill unit and hand-piece (Implantmed SI-923 Dental drill unit, Handpiece WI-75E/KM 20:1, W&H Nordic, Täby, Sweden) with a drilling speed set at 2,000 rpm was used. To simulate the clinical situation, freehand drilling was performed. When

irrigation was used, tap water (22°C) was perfused manually with a 20-cc syringe.

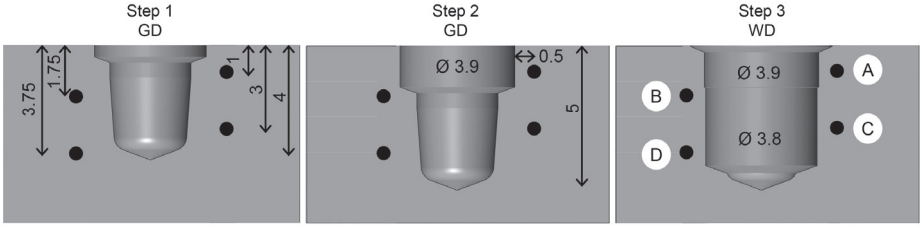

Figure 15 *Thermocouple position in the three different drilling steps, exemplified here for the MIPS system (all distances in mm). In the last step, all the probes are positioned 0.5 mm from the drill tract. The dots indicate the positions of the thermocouples in relation to the osteotomy (in Step 3, indicated as A, B, C, and D.*

After drilling, the blocks were scanned (Zeiss Metrotom 800 CT, Carl Zeiss Industrielle Messtechnik GmbH, Oberkochen, Germany) to determine the exact distance between the thermocouple canals and the final osteotomy walls. Using this exact distance, a curve-fit was applied to the data compensating for the effect of an erroneous (distance≠0.5 mm) distance between probe and the osteotomy wall.

3.6.4 Histology and histomorphometry

The implants and surrounding bone were retrieved *en bloc* and processed by fixation in 4% paraformaldehyde, stepwise dehydration in a graded ethanol series, followed by embedding in plastic resin (LR White, London Resin Co. Ltd, UK). The embedded blocks were bisected. One half-block of each specimen was used to prepare a 50-µm thick central ground section (EXAKT1Apparatebau GmbH & Co, Norderstedt, Germany) [28] stained with toluidine blue. Qualitative histology and quantitative histomorphometry were performed, to determine the amount of bone-to-implant contact (BIC) and bone area (BA) within the implant threads, using light optical microscopy (Nikon Eclipse E600; Nikon NIS-Elements software) (Paper III).

3.6.5 BSE-SEM and resin cast

In Study III, the embedded resin blocks were subjected to backscattered electron scanning electron microscopy (BSE-SEM) imaging, to determine the mineralised bone area and the osteocyte density, i.e. the average number of osteocytes per mineralised surface, was determined [Paper III]. Using high-vacuum secondary electron SEM imaging, the same resin-embedded bone-implant blocks were used for the direct visualisation of osteocytes adjacent to the implant surface using a resin cast etching procedure [Paper III].

3.6.6 Raman spectroscopy

In Study III, Raman spectroscopy was used to investigate the composition of the newly formed bone within and around the first implant thread filled with new bone, below the level of the original cortical bone. Spectra were recorded in areas of mineralised bone, approximately 50-100 μm from the implant surface at the inner 1/3 (thread valley), the outer 2/3 (thread flank) and immediately outside the thread.

3.6.7 Ultrastructural analysis

One selected tissue block from the laser-modified group in Study III was polished and coated with aluminium and palladium and transferred to focused ion beam (FIB) equipment for the preparation of an electron transparent lamella for subsequent transmission electron microscopy (TEM). Bright-field TEM and high-angle annular dark-field scanning TEM (HAADF-STEM) were performed to study the ultrastructure of the bone-implant interface. Further, site-specific, energy-dispersive X-ray spectroscopy (EDX) was performed across the interface zone using a nanoprobe in STEM mode.

3.6.8 Microbiology

In Study V, three different sampling procedures (the abutment, the peri-abutment exudate and the soft tissue) were employed for the identification and quantification of colonising bacteria, from baseline up to 12 months, using quantitative culturing. Total viability counts of colony-forming units (CFU) of aerobic and anaerobic bacteria were measured on the abutment (CFU/abutment), paper-point (CFU/paper-point) and in soft-tissue samples (CFU/biopsy). Additionally, staphylococci, enterococci, *Escherichia coli* and *Pseudomonas aeruginosa* were quantitated using selective media.

Briefly, the soft-tissue biopsies were homogenized, the paper-points vortexed, and the abutments both sonicated and vortexed in order to extract the bacteria. Diluted and undiluted homogenised samples were spread on duplicate agar plates of the following media: 5% horse blood Columbia agar (for aerobic bacteria), Brucella agar (for anaerobic bacteria), staphylococci agar, enterococci agar, and CHROMagar™ Orientation (CHROMagar, Paris, France) (for *E. coli* and *P. aeruginosa*). All plates, except Brucella, were incubated aerobically at 37°C and 5% CO_2 for two days until colonies were counted. Brucella plates were incubated under anaerobic conditions at 37°C for five days before CFU counting. In order to increase detection, enrichment of the samples was performed by culturing an undiluted homogenised specimen in one thioglycolate broth tube (TAS) and incubated under aerobic conditions for five days. The TAS tube was read (positive/negative growth) and re-plated in

the event of negative agar cultures. The biochemical identification of staphylococcal species was performed using the API Staph Strip system (bioMérieux SA, Montalïeu-Vercieu, France).

3.6.9 Clinical intra-operative outcome

For the evaluation of surgical technique [Papers VI, VII, VIII], intra-operative data such as surgical time, deviations from instructions, complications and adverse events, as well as any issues experienced with the instruments, were collected.

3.6.10 Clinical post-operative outcome

For the assessment of skin reactions, the Holgers score was used in all clinical studies. The Holgers score is a macroscopic peri-abutment skin assessment scale where 0 = no irritation, 1 = slight redness, 2 = red and slightly moist tissue, no granuloma formation, 3 = reddish and moist; sometimes granulation tissue and 4 = removal of skin-penetrating implant necessary due to infection [115]. In Study VIII, the primary outcome variable was the incidence of inflammation, defined as a Holgers score of ≥ 2, as this often requires substantial treatment (e.g. systemic antibiotics or local intervention). Other outcomes included wound healing, presence of dehiscence after surgery, soft-tissue height, loss of skin sensibility, pain, cosmetic results, hygiene, ISQ-values and implant extrusion rate. Any adverse events were registered.

3.7 Statistical analysis

In Study III, the Mann-Whitney U test was used for the statistical comparison of implant surface characterisation [Paper III] and Wilcoxon's signed-rank test was used to compare the biomechanical outcome (RFA and RTQ), histomorphometry (BIC and BA) and Raman spectroscopy.

In Study IV, statistical comparisons of the cutting performance (force and torque) of the drill systems were made using the Mann-Whitney U test. For differences in heat generation during drilling, the independent-samples t-test was used for comparisons of drill systems, whereas one-way ANOVA was used for the comparison of drilling procedures within the same drill system. A three-way mixed-model ANOVA was run to evaluate the effect of the two drill systems (between-subject factor) on heat generation using drilling procedure and thermocouple position as within-subject factors and including the interaction among factors.

In Study V, an independent-samples t-test was used for the comparison between test and control groups with respect to chemical and topographical

surface parameters, mean CFU count at the three compartments at three months, the mean CFU between time points (three and 12 months) and for the distribution of the Holgers score over observations. One-way ANOVA was employed for the comparison of CFU/tissue biopsy between the three time-points (baseline, three months and 12 months) and for the comparison between the three sampling methods at each time point. The chi-square test was used to compare the clinical outcome (Holgers score, pain, hygiene) between groups and pooled between time points (three and 12 months).

In Study VI, an independent t-test was used to compare the surgical time per case performed by the same surgeon.

In the clinical studies, VII and VII, the primary endpoint was described by comparing the proportions of inflammation (Holgers score ≥ 2) between surgery and the three-month follow-up using a 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. The extrusion rate was compared using the log-rank test.

In addition, correlation analyses were performed when applicable. Pearson's correlation and linear regression models were used to determine the relationship between different parameters of osseointegration (RTQ, BIC, BA, ISQ) [Paper III]. Pearson's correlation was used to evaluate the relationship between clinical and microbiological data, using both test and control groups as separate and pooled [Paper V].

Statistical analyses were performed using SPSS (IBM Corporation, USA) [Papers III-VI], or conducted by Statistiska Konsultgruppen (Gothenburg, Sweden) [Papers VII, VIII]. Significance were considered for p values of < 0.05.

3.8 Ethical considerations

Study I was reviewed and approved by the Norwegian Animal Research Authority (Forsøksdyrutvalget, Brumunddal, Norway). Studies II and III were reviewed and approved by the Regional Animal Ethics Committee, Gothenburg, Sweden (Dnr 325-2007 and Dnr 291-2012, respectively).

The procedures in Study VI were in accordance with local legislation within the individual countries participating in the evaluation. Approval was not

required from an ethics committee, because clinical data were readily available and were used after being rendered anonymous. The clinical studies, V, VII and VIII, were performed in accordance with ISO 14155:2011 and the Declaration of Helsinki (Washington 2002). Each patient was thoroughly informed, both verbally and in written form, of all the procedures and requirements of the study to which they were recruited. All patients included in the studies provided written informed consent. Both studies were sponsored by Oticon Medical AB (Askim, Sweden). The investigators had full access to all data. For the reporting of Study VIII, the CONSORT guidelines were followed. Study V; this study was approved by the Regional Ethical Review Board in Stockholm, Sweden (2014/1566-31/2), and registered at clinicaltrials.gov (NCT02304692). Monitoring was provided by the sponsor. Studies VII, VIII: this study was approved by the ethics committee at the Maastricht University Medical Centre+ (NL50072.068.14) and registered at ClinicalTrials.gov NCT02438618. Data analysis was conducted by Statistiska Konsultgruppen (Gothenburg, Sweden). Monitoring was performed by the sponsor and TFS Develop (Zaltbommel, The Netherlands).

4 RESULTS

4.1 Paper I

This study explores the opportunity to integrate a percutaneous implant fitted with an internal mesh structure with a porcine small bowel.

The implants were retrieved according to plan after one to four weeks. Two stoma port implants were lost two days after surgery due to the rupture of the abdominal incision. At retrieval, abscess formation and fistulas were present in four of the eleven stoma ports.

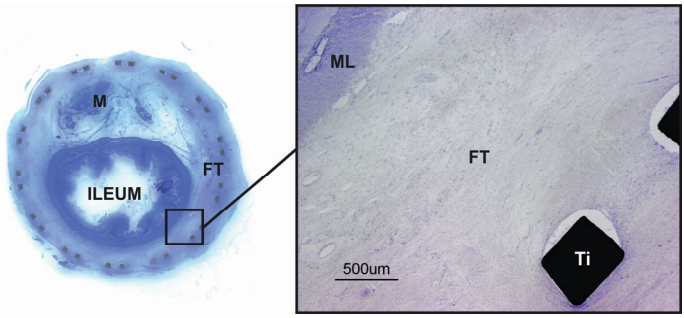

Figure 16 *Histological micrograph cross-section of the stoma port and surrounding tissue. The mesentery (M) and fibrous tissue (FT) fills up the space inside the port. A mild inflammatory reaction, mainly consisting of scattered inflammatory cells, is found throughout the fibrous tissue. The ileum wall with its outer muscle layer (ML) is merged with the FT.*

One major problem during the experiments was self-inflicted trauma to the implant and the efferent ileum segment. For one of the stoma port a histological evaluation revealed that the mesh was incorporated in well-vascularised connective tissue without evidence of inflammation (Figure 16). This fibrous connective tissue had merged with the muscularis externa of the ileum segment inside the stoma port. For the remaining ports, however, a histological evaluation demonstrated a low degree of ingrowth in the mesh structure and insufficient anchorage of the ileum inside the ports. The tissue around the implants was associated with a medium to high degree of inflammation and areas of exudate. Areas with infiltration of polymorphonuclear neutrophils and lymphocytes suggested a bacterially driven inflammation. Morphologically, aggregates of cocci, suggesting biofilm formation of parts of the implants, were detected.

4.2 Paper II

In this study, a soft-tissue-anchored, percutaneous port, used as a mechanical continence-preserving valve in ileo- and urostomies, was evaluated in dogs. After implanting the stoma port and constructing an ileal reservoir, seven of eight dogs were fit for conversion to a functioning ileostomy or urostomy. During follow-up (median 13 weeks), the skin failed to attach to the implant, but the intestine inside the stoma port appeared to be attached to the mesh. After reaching adequate reservoir volume, the urostomies were made continent by attaching a lid to the implant. The experiments were ended at different time intervals due to implant-related adverse events, such as pyelitis, an ischemic ileum and abscesses.

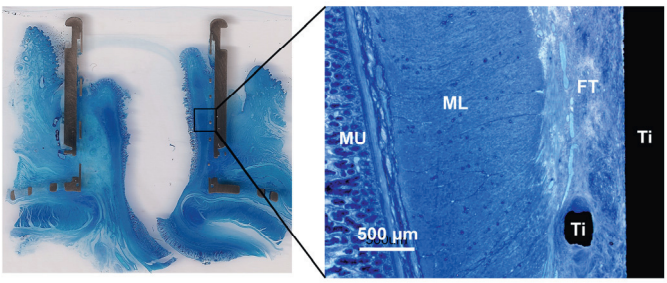

Figure 17 *Light micrograph showing the integration between the intestine and the inner mesh and cylindrical surface of the titanium port in the ileostomy model. The vascularized fibrous tissue (FT), fills the area around the mesh structure and merges with the ileum. MU=ileum mucosa, ML=muscularis, Ti=titanium.*

In only one case (ileostomy model) did the histological evaluation reveal integration at both the implant-intestine and implant-skin interface, with a low degree of inflammation and the absence of bacterial colonisation (Figure 17). For the remainder, integration was not obtained or maintained. Instead, the morphology was characterised by mucosal downgrowth and biofilm formation. Integration was more frequent in the mesenteric portion of the intestinal circumference compared with the anti-mesenteric side. The skin-implant junction was characterised by the absence of direct contact between the epidermis and the implant. Varying degrees of epidermal downgrowth, granulation tissue formation, inflammatory cell infiltration and the presence of bacteria and biofilm were prominent findings. In contrast, the subcutaneously located anchoring part of the titanium port was well integrated and encapsulated by fibrous tissue.

Taken together, the results of the Studies I and II show that it is possible to achieve integration between a soft-tissue-anchored titanium port, skin and intestine. However, predictable long-term function could not be achieved in these animal models, due to implant- and non-implant-related adverse events.

4.3 Paper III

In this study, the early bone response to screw-shaped titanium implants, with and without selective laser-modification, placed in rabbit tibia for eight weeks, was evaluated in terms of bone growth, ultrastructure and biomechanical anchorage. Machined titanium implants were treated in the thread valleys using an Nd:YAG laser. Compared with the relatively smooth, untreated machined implants, this resulted in a distinct hierarchical structure with a combined macro- and microtopography with a superimposed nanotexture, all confined to the thread valley. The increase in surface roughness was confirmed with an interferometer. An increased oxide thickness, measured using AES, was revealed for the laser-modified surface (53 nm) compared with the machined surface (13.8 nm). Further, the AES analysis confirmed similar surface chemistry (C, Ti and O) for the two types of implants.

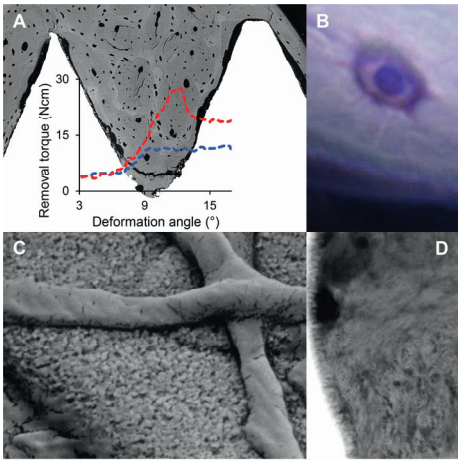

Figure 18 *A graphical abstract of the biomechanical (A), osteocyte (B, C) and ultrastructural (D) findings in Paper III.*

After eight weeks, the RTQ for the laser-modified implants was 153% higher than that of the machined implants (Figure 18A). The ISQ values increased from installation to retrieval for both implant types. An equally large bone area (BA) and bone-to-implant contact (BIC) was recorded for both implant types. During RTQ measurement, the load deformation curve revealed distinctly different failure patterns. The machined implant showed a moderate torque increase, followed by a plateau. In contrast, the laser implant revealed a sharp torque increase, followed by a distinct breakpoint with a shorter or no plateau period (Figure 18A).

Histological analysis showed osteocytes within a few micrometres of the implant surface (Figure 18B). Using SEM on resin cast etched blocks, osteocyte canaliculi appeared to directly approach the laser surface (Figure 18C). Using TEM, mineralised collagen fibrils were aligned parallel to the laser surface, interlocked with the laser-induced thick oxide, providing evidence of direct bone-bonding (Figure 18D). As observed for the laser-modified implant with back scatter SEM, the newly formed bone within the implant thread was highly mineralised. Raman spectroscopy indicated similar bone mineral crystallinity, mineral-to-matrix and carbonate-to-phosphate

ratios for both implant type. Correlation analyses revealed a strong positive correlation for the RTQ with BIC in the thread valley. The ISQ correlated with the amount of bone growth around the implant. Taken as a whole, the results suggested that RTQ had the highest sensitivity to measure implant stability and that the increased stability of the laser-modified implant is attributable to direct bone bonding with the site of laser modification.

4.4 Paper IV

In this study two drill systems for osteotomy site preparation for the installation of bone-anchored hearing implants were evaluated and compared with respect to cutting performance, heat generation, drilling procedure and distortion of the bone during *ex vivo* drilling.

At a constant feed rate of 1mm/s the mechanical evaluation of the cutting performance demonstrated that less force was required to drill into the artificial bone using the MIPS guide drill and widening drill compared with the corresponding guide drill and countersink of the conventional Ponto system. Computing the energy needed to generate the osteotomy it was revealed that the mean thrust energy was significantly lower for both MIPS drills compared with the corresponding Ponto drills. For guide drilling, mean work was 33.72 (1.21) Nmm and 43.64 (3.90) Nmm for the MIPS and Ponto systems, respectively ($p < 0.001$). The corresponding scores for the second drill step were 5.25 (0.34) Nmm and 16.37 (6.43) Nmm for the MIPS and Ponto system respectively ($p < 0.001$). In contrast, the energy related to the torque was a factor of 1,000 times higher compared with the thrust energy. The mean torque energy was more than twice as high for the MIPS guide drill, with a score of 17.56 (1.17) Nm, compared with the Ponto guide drill, with 7.69 (0.23) Nm ($p < 0.001$). For the subsequent step, the difference in torque energy between MIPS and Ponto was not statistically significant ($p = 0.106$).

When drilling according to the clinically recommended standard procedure (direct drilling with continuous irrigation), the temperature increase was significantly higher for the MIPS system compared with the Ponto (MIPS 5.5 °C *vs* Ponto 2.4 °C) (Figure 19). On the other hand, a significantly lower temperature increase was demonstrated for MIPS (MIPS 4.3 °C *vs* Ponto 9.0 °C) when an impaired irrigation procedure was applied. The results also show that when drilling is prolonged (drill bit left idling after reaching full depth), the temperature increases significantly for both systems compared with recommended standard procedure. However, the heat generation during an idling drilling procedure was significantly higher for MIPS (16.3 °C *vs* Ponto 6.8 °C) (Figure 19). A three-way mixed ANOVA illustrated a statistically significant three-way interaction between drill systems, drilling protocols and

position. The histological evaluation showed relatively more even cut surfaces and fewer micro cracks in the osteotomy wall when using MIPS compared with the Ponto system.

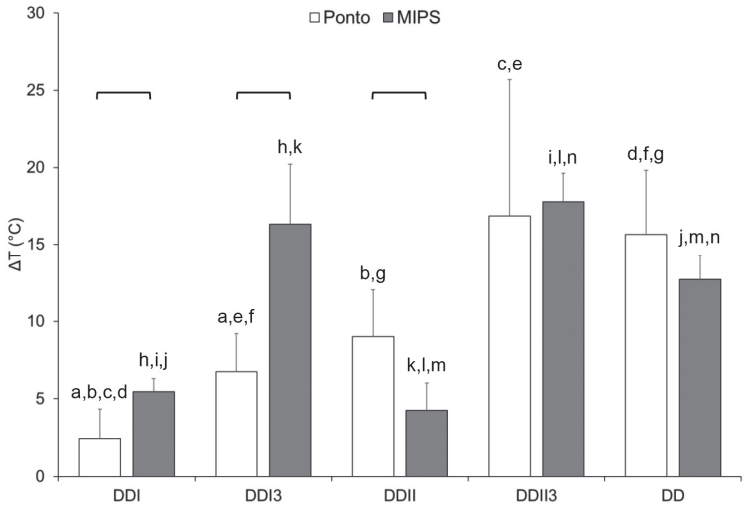

Figure 19 *Graph showing the mean maximum temperature increase at the position with the highest mean temperature increase for each combination of drill system (Ponto versus MIPS) and drilling procedure. Brackets indicate statistically significant differences between Ponto and MIPS systems within the same protocol (T-test, $p<0.05$). Letters indicate statistically significant differences between different protocols within the same drill system (one-way Anova, $p<0.05$). No correction for multiple comparison was made. See Table 4 for a description of the drilling protocols.*

Within the limits of the present *in vitro* study, the results show that multiple factors influence the distribution of heat as well as the level of the temperature increase at the site of the osteotomy. The results also demonstrate that altering the drill design influences the mechanical performance as well as the degree of heat generation. Although drill bits with a twist drill design in combination with a guided drilling approach, generated relatively more heat, especially during a prolonged drilling procedure, it is more forgiving in the case of impaired irrigation. In conclusion, this study suggests that the present MIPS system for a flapless approach, conveys a promising design for an efficient yet safe osteotomy site preparation for BAHS installation.

4.5 Paper V

In this study, abutments with different topologies were evaluated and compared with respect to the clinical outcome and microbiological profile. Further, three different sampling methods for the identification and quantification of colonising bacteria were evaluated.

The clinical outcome measures (Holgers score, hygiene, pain, numbness and implant stability; ISQ) did not differ significantly between test and control after three and 12 months. The comparisons between three and 12 months for each clinical outcome measure were performed on pooled data and did not reveal any significant difference. Sampling from three different compartments (peri-abutment soft tissue, peri-abutment fluid space and retrieved abutment) enabled the isolation and quantitative determination of the number of viable bacteria and the presence of potential microbial pathogens of the skin-penetrating BAHS. At baseline, the soft tissue was mainly colonised by anaerobic bacteria, and anaerobic bacteria were subsequently detected in all three compartments at three and twelve months (Figure 20). In the peri-abutment space exclusively, three months after the installation of the abutment, a significantly higher number of anaerobes, aerobes and staphylococci were demonstrated for the polished *vs.* the machined abutments. The quantity of aerobic bacteria in the tissue biopsies increased significantly between baseline and three months, whereas no significant temporal change was shown for the rest of the samples and bacterial groups.

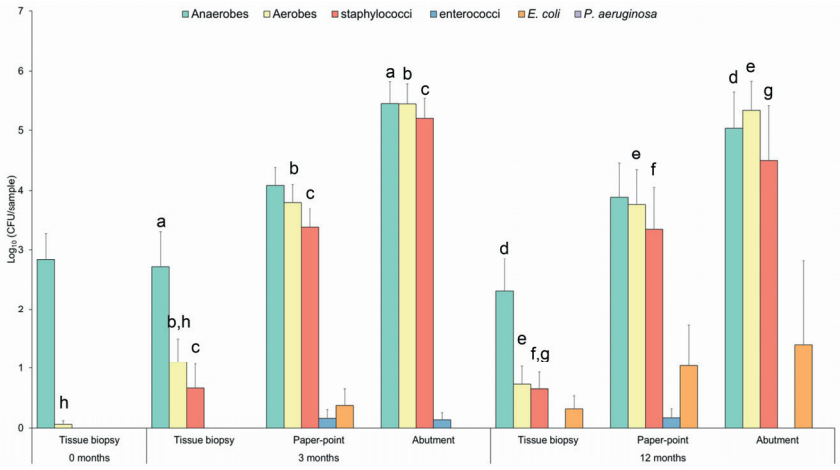

Figure 20 *Total viable counts of aerobic and anaerobic bacteria as well as aerobic bacterial species (staphylococci, enterococci, Escherichia coli, and Pseudomonas aeruginosa) at baseline (n = 12), three months (n = 12), and 12 months (n = 9 biopsies and paper-points; and n = 4 abutments). Data represent the mean ± SEM. Bars that share the same letters are significantly different p < 0.05*

Staphylococcus spp. were not identified at baseline, but they were found in all compartments at both 3 and 12 months after implantation (Figure 20). The biochemical identification of the isolated staphylococcal colonies revealed that several of the patients were colonised by the same species, and probably by the same strain, over the first year of BAHS implantation. The common skin coloniser, *S. epidermidis* was identified in all patients but one (11/12), whereas the potentially harmful pathogen, *S. aureus,* was isolated in five of the twelve patients.

Despite the small patient number, several associations between the clinical outcome and the microbiological parameters were found. The Holgers and pain scores at three months post-implantation correlated with each other and with the number of aerobes in the tissue prior to implantation. Taken together, ahead of a large clinical trial, the present pilot trial largely confirmed a suitable study design, sampling and analytical methodology to determine the effects of modified abutment properties.

4.6 Paper VI

In this multicentre clinical evaluation, the use of MIPS to install a bone-anchored hearing system was evaluated with respect to intra-operative results and short-term post-surgical outcome.

Seventy-seven implants were installed in 76 adult patients. In two cases, conversion to a linear incision technique was necessary. The mean time for surgery, from skin punch to healing cap, was 16 min (median 13 min). There was a statistically significant reduction in surgical time per case with increased numbers of MIPS cases performed by the same surgeon. The average time taken per surgery per surgeon dropped from 21 to 12 min ($p < 0.001$) after performing ≥ 2 surgeries. In 74.0% (57/77) of the surgeries, no intra-operative events were reported. There was an intra-operative cerebrospinal fluid (CSF) leak in one of the cases with an exposed dura.

Post-operative results were collected from a total of 160 follow-up visits. The median time following surgery was 34 weeks (range 20-49 weeks). Implant survival was 74/77 (96.1%), with three implant losses recorded. The rate of adverse soft-tissue reactions (Holgers ≥ 2) was 5.0% (eight of 160 visits) and 9.2% (seven of 76 implants) per visit and per implant respectively. Two cases of Holgers 3 were registered and they subsequently resolved with local treatment. Overall, from 160 follow-up visits, numbness and a general sensation of pain were reported by the patients in 3.1% and 9.4% of the visits respectively.

4.7 Papers VII and VIII

This multicentre, open, randomised, controlled clinical trial compared the outcomes three months after BAHS installation using MIPS (test) and the linear incision technique with soft-tissue preservation (control). The research and study protocol for this trial is described in Paper VI, whereas the clinical results after three months follow-up are reported in Paper VII.

Sixty-three subjects were included in the analysis, with thirty-three subjects randomised to the test group (52%) and thirty to the control group (48%). The patient characteristics were similar between the groups. Intra-operative events were few in number and comparable between the two groups. The surgical procedure time was significantly shorter in the test group compared with the control group (6.52 vs. 13.3 minutes). The incidence of inflammation (Holgers score ≥ 2) between surgery and 12 weeks showed no statistically significant difference between surgical techniques (Figure 21A). Loss of sensibility was significantly less in the test group compared with the control group on all follow-up visits (Figure 21B). No significant differences in pain scores were observed. The cosmetic outcome parameters (natural skin position, extent of baldness, scarring, skin colour and indentation, overall observer scores) were all significantly better in the test group compared with the control group. However, there was no difference between the groups when the subject him/herself scored satisfaction with cosmetics.

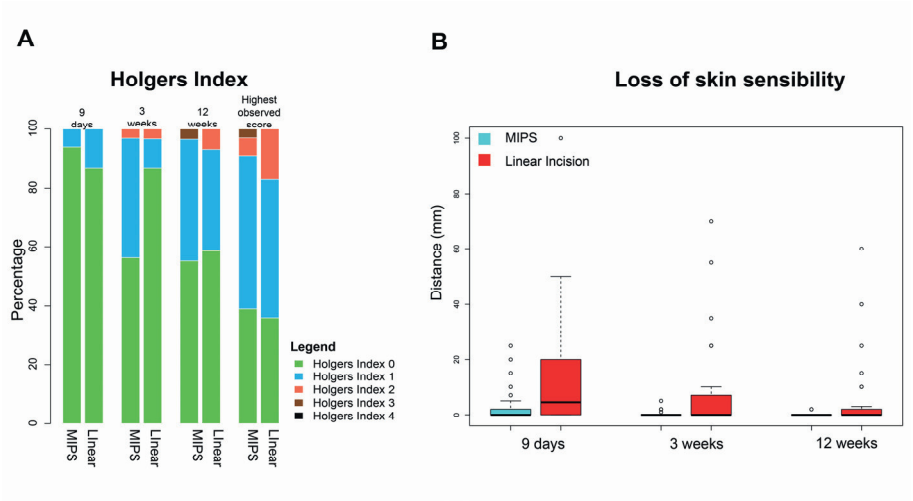

Figure 21 *Stacked bar chart for the Holgers Index scores on standard follow-up visits and the highest observed Holgers Index score (A). Box plots of loss of skin sensibility per treatment group on standard follow-up visits (B).*

The ISQ was significantly influenced by the surgical technique, abutment length and time. In the test group, ISQ High was 2.35 points lower compared with the control group. In comparison, abutment length influenced ISQ values by 6-12 points. 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%) (non-significant difference). Implant loss occurred between 25 days and 90 days post-surgery. No obvious association was observed between the initial ISQ and implant loss.

In conclusion, there were no significant differences in adverse skin reactions between the MIPS and the linear incision technique. 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.

5 DISCUSSION

5.1 Soft-tissue-anchored stoma implant

Few techniques for creating continent enterotomies using either external removable devices or implants have been reported and fewer still remain in clinical practice [126, 241, 242]. Other approaches to achieve a stoma free solution are currently in use, such as the ileorectal anastomosis. In contrast to the stoma ports evaluated in Papers I and II, none of the historical attempts required the bowel wall to integrate with a device. To the author's knowledge, there are no previous reports of permanent implants that integrate with the intestine. Nevertheless, the short-term outcome when using a subcutaneously positioned ring made of polyethylene terephthalate mesh to prevent parastomal hernia after stoma surgery was recently reported [243]. It appears that part of the efferent segment of the ileum or colon is in fact in contact with the mesh, but data regarding tissue reaction and possible tissue integration are unavailable. To date, thirty patients have been implanted and followed for 30 days, without any reported complications [243].

During the initial phase of healing around implanted foreign materials there is a competition for the surface between different cell types and microorganisms [72]. The provision of an optimal healing environment at the implant surfaces therefore calls for careful attention. A prerequisite for a functional skin-penetrating stoma implant is the creation of a structural barrier between the intestine and the surface of the implant. If bacteria and faecal debris are able to invade the space between the intestine and the implant surface, a barrier of this kind would be disturbed. In Study II, only one of the implants achieved complete integration of the bowel with the implant. It is likely that the formation of a structural and functional inner barrier was not obtained or maintained for the remaining implants illustrating the race for the surface [72]. Evidently, the detected bacteria and biofilm formation on many of the implants [Papers I, II] highlights the fact that measures to minimise bacterial contamination at the interfaces during healing are important.

The visceral serosa that lines the internal organs, including the bowel, comprises a thin layer of loose connective tissue covered by a single layer of mesothelial cells. The main functions of the mesothelium are to provide a protective barrier and an adhesion-preventing surface for the free, frictionless movement of apposing organs in the abdominal cavity by the continuous release of serous fluid [244]. The mesothelium plays a role in fluid and cell transport, inflammation and tissue repair and the mesothelial cells have been shown to transdifferentiate [136, 245]. Moreover, mesothelial cells modulate

serosal repair and inflammation through their ability to synthesise cytokines/chemokines, growth factors, extra-cellular matrix proteins and intracellular adhesion molecules [246]. Importantly, repair occurs diffusely through the injured mesothelial membrane and not from the wound edges, as in the case of epithelial organs and tissues [244]. A property of this kind would make a precise adaptation to a titanium surface even more difficult. The hypothesis was to use a mesh inside the lumen of the implant to stimulate the development of fibrous tissue, thereby facilitating the anchorage and adhesion of the bowel wall to the implant. Interestingly, the mesothelium could not be identified in the histological slides [Paper II]. Instead, the muscularis was directly connected to the fibrous tissue covering the mesh structure, thereby creating the opportunity for the adhesion and anchorage of the ileum inside the implant. Further, integration between the titanium port and the intestine was more commonly observed in the mesenteric region of the intestinal circumference, whereas mucosal downgrowth and biofilm were more commonly associated with the non-mesenteric portion [Paper II]. At present, it is difficult to determine the cause and effect behind these observations. Mechanical stresses were exerted on the tissue-material interface due to the intestinal propulsion as well as the daily catheterisation of the stoma during the experiments. Further, the mesenteric fatty tissue might serve as a cushion, thereby protecting from repeated mechanical stresses at the tissue-mesh interface, a factor that might favour ingrowth on this side of the port. The role of the mesothelium and the vascularised mesenteric connective tissue is more difficult to elucidate. More basic experimental work is needed to further understand the role of the mesothelium in the tissue response between the titanium mesh and the serosa.

A second prerequisite for soft-tissue-anchored percutaneous implants is the formation of a structural and functional barrier between skin and implant [85]. The epithelial downgrowth and pocket formation allow bacterial invasion to occur between the skin and the implant interface, which often results in infection [59, 79, 247]. One important finding in the present studies in both experimental models was the difficulty involved in achieving a skin-implant integration [Papers I, II]. One important conclusion, was that the implant and implant area could not be sufficiently shielded from self-inflicted trauma and excessive movements. As a result, the stress at the skin interface was substantial resulting in granulation tissue, epidermal downgrowth and biofilm. These stresses might be more controlled in humans. In fact, a titanium stoma port modified to fit the human small bowel was trialled in four patients [248]. In this modification only the mesh structure penetrated the skin, with the purpose of merging skin and intestine through this mesh. Problems similar to those in the present experimental models were reported such as insufficient tissue ingrowth, necrosis and efferent ileum retraction leading to faecal leakage

(three patients) and revision surgery (two patients). One patient used the lid permanently to maintain stoma continence, one used it partly together with a stoma bag, whereas the remaining two patients used stoma bags. After 18 months, one device was explanted due to leakage. Interestingly, no infections were reported. The authors concluded that bridging the connective tissue between the intestine and skin is crucial and that further development of the cap, the implant and the implantation method is necessary.

The results of the present experimental studies indicate that a titanium implant can be used as a port for an enterostoma to create continence. Nevertheless, in order to be able to create a predictable, long-term clinical solution using a principle of this kind, it is necessary to improve the current techniques. The main strategies include the reduction of tissue mobility by optimising the surgical procedures, port design and accessories, the exploration of a longer healing phase to allow complete integration and the reduction/delay of the post-operative bacterial burden. Unless barriers at both implant-skin and implant-intestine junctions are created, epidermal and mucosal downward migration and biofilm formation will jeopardise implant performance.

5.2 The role of implant material and design

For endosseous implants the main drivers, particularly within dental implantology, for the continued development of surface modifications, shape, material and type of implants, have been two-fold: to reduce the loss rate, even for patients with soft and compromised bone qualities [32], and to reduce the loading time [249], i.e. the time patients must wait after primary surgery until they can benefit from the treatment. Nevertheless, according to evidence-based, Cochrane Systematic Reviews, little or no difference in clinical performance is found when reviewing the literature for randomised, controlled trials of different dental implant systems and protocols [249-251]. In 2010, Palmquist et al. found that randomised, controlled studies in the dental field revealed no scientific evidence that any particular type of established dental implant surface has superior long-term success [18]. It is likely that modified and rougher surfaces used for dental implants will produce an improved outcome, mainly for implants placed in the trabecular bone of the maxilla, as the success rate there is lower compared with the mandible. There are also indications of improved clinical results in the maxilla, whereas, in the cortical bone of the mandible, the clinical success is very high and similar to that of any of the available titanium implant surface modifications.

5.2.1 Design

Increasing the width of the osseointegrated implant is one method to enhance stability. Biomechanically, when placed in an appropriately sized osteotomy, the engagement of a wide-diameter implant in the bone is increased and the surface available for osseointegration is enlarged [252, 253], with improved survival rates as a result [254]. In the field of BAHS, 4.5-mm wide diameter implants, with and without surface modification, were introduced some years ago, replacing the conventional 3.75-mm implants with machined surfaces. Superior stability, in terms of ISQ value, at installation and throughout follow-up has been demonstrated both experimentally [255] and clinically [174, 213]. No improvement in survival rate in adult patients was seen after the five-year follow-up [174], but since the loss rates for adult patients are low, the power of the studies is unable to answer that question. In contrast, for paediatric populations, where implant loss rates are typically higher, an improved survival rate has been shown for a wide-diameter implant with a blasted surface compared with historical data when using narrower machined implants [30].

The macroscopic geometry, design and cutting characteristics of the threads of an implant, as well as the osteotomy-to-implant diameter ratio, are most certainly important features biomechanically, whereas it is more uncertain whether they will make any difference to osseointegration. For BAHS, only two types of wide diameter implant exist in the marketplace and a direct comparison, experimentally or clinically, has never been performed. Admittedly, these studies are difficult to design, especially clinically. Accordingly, a review of randomised, controlled studies of different dental implant types failed to reveal any difference in clinical outcome [251].

5.2.2 Implant surface modification

The modification of implant surfaces aims to improve bone healing and biomechanical fixation by altering the physicochemical properties [18, 23, 38]. This can be achieved by changing the surface topography, on either the micro- or the nanoscale, or by modifying the surface chemistry. It is currently understood that even nanotopographical features encompassing the atomic, molecular and macromolecular length scales act as modulators of cellular behaviour [256]. Moreover, nanoscale structures have also been shown to stimulate osseointegration *in vivo* [43].

In Study III, a surface-modified implant designed to be implemented to improve the BAHS was evaluated experimentally in comparison with a similar implant with a machined surface. Bone tissue can be regarded as a highly hierarchical material. Using selective laser ablation, increased surface roughness exhibiting a dual micro- and nano roughness, mimicking this

hierarchy, is obtained. A limited increase in the titanium oxide thickness is also generated. During the process, the melting and re-solidification of the metal on the surface creates microscale (1-10 μm) globular structures. They represent the cellular length scale and provide a large surface area for the ingrowth of bone, as well as mechanical interlocking. Superimposed on the microstructure are nanoscale features with a coral-like arrangement. They represent the length scale of the minerals and collagen bundles in the bone.

After eight weeks of healing in the rabbit [Paper III], laser-modified implants exhibit enhanced biomechanical anchorage compared with machined implants, as measured by RTQ (removal torque). The load deformation pattern suggests a fracture-like deformation for the laser-modified implants. This is supported by histological and BSE-SEM observations of fracture lines in the bone in the laser modified thread valleys. In contrast, the gradual separation of the bone from the implant surface is evident for the machined implants. Bone-to-implant contact (BIC), bone area in threads (BA), extracellular matrix composition and osteocyte densities were similar for the machined and laser-modified implants. Collectively, these outcomes most probably did not constitute determinants of the large difference observed in RTQ between the two implant types.

The observation of well-mineralised collagen fibrils aligned closely and parallel to the micro-scale surface contour of the laser-modified implant surface could be a clue to the strong biomechanical anchorage. This ultrastructural observation corroborates previous observations of laser-modified implants extracted from humans [16, 257] and animals [14, 258]. The result of a retrospective clinical study of dental implants, with a laser-modified surface, shows a cumulative survival rate of 99.3% for 310 implants placed in 83 patients after a five-year period [259]. The first short-term clinical results from using a modified surface of this kind for hearing implants was evaluated retrospectively in 34 adult patients at three centres after one year [260]. Excellent survival rates, good soft-tissue tolerability and few complications were reported. However, prospective long-term studies in adult and paediatric patients with compromised bone are needed in order fully to evaluate the possible benefits.

It is concluded that selective laser ablation to generate a specific combined micro and nano roughness in the thread valley promotes stronger integration in bone and improved biomechanical anchorage during the early period of osseointegration *in vivo,* in comparison with machined implants.

5.2.3 Implant stability

Implant stability has repeatedly been acknowledged as a major factor in the survival of osseointegrated implants, because implant micromotion is believed to be one of the factors responsible for the failure of osseointegration. The main determinants of implant (primary) stability are (i) the mechanical properties of the bone tissue at the implant site and (ii) how well the implant is engaged with the bone tissue [261]. Unsurprisingly, in the rabbit study [Paper III], there was no difference in ISQ at insertion between the groups, as the same site, drilling protocol and macroscopic implant geometry were used for both implant types. Further, there was no difference in ISQ between the laser-modified and the machined implants after eight weeks of healing. Moreover, there were no differences in BIC and BA between the groups. These findings indicate that RTQ had greater sensitivity to measure implant stability compared with ISQ. In a prospective study of 195 dental implants the relationship between ISQ value and a number of bone and implant related features was evaluated [262]. Longer, wider implants achieved higher primary stability than shorter, narrower implants. However, these correlations lost their significance after osseointegration had taken place, indicating the limitations of the ISQ measurement to distinguish the degree of osseointegration. For bone-anchored hearing implants, ISQ is commonly used to assess the stability of the system. For these systems, it has been shown that primary stability is influenced by abutment length, bone quality and degree of seating [263]. Caution is therefore warranted when it comes to interpreting absolute stand-alone ISQ values individually [264].

Interestingly, in the study comparing MIPS surgery with a conventional linear incision technique [Paper VIII], the ISQ was significantly influenced by the surgical technique. Primary ISQ High values were about 2.4 points lower in the MIPS group compared with the control group. This small difference has likely no clinical impact. In contrast, the opposite has been observed for dental implants where the flapless procedure demonstrated slightly favourable primary ISQ values, compared with the open method [262, 265]. In none of the articles was an explanation of this difference provided. In the case of MIPS versus linear incision, it is important to recognise that the dimensions of the osteotomy in the proximal cortical area are slightly larger for MIPS compared with the conventional drilling system. Clinical studies have shown that the cortical thickness is strongly correlated to an increase in primary ISQ [262]. It is possible that the 0.1 mm larger diameter in the proximal cortical part for MIPS results in the same effect, as the RFA is sensitive to the anchorage of the implant in that region.

5.2.4 Abutment surface modification

For a percutaneous system, the assumption has been that a seal between the soft tissues and the implant is necessary to avoid epithelial downgrowth and subsequent infection [85]. Multiple strategies to promote the soft-tissue attachment around a percutaneous implant have been evaluated experimentally and, in rare cases, clinically [266]. The strategy will clearly vary depending on application, design, use and anatomical position. Examples of strategies are the choice of material (metal or polymers), surface topography modification (rough, smooth, porous, grooved), surface chemistry, design and surgical approach [266]. One approach is to incorporate a porous structure in the dermal region of the percutaneous abutment in order to optimise the integration and therefore inhibit epithelial downgrowth. Animal experiments demonstrate promising outcome short-term, however the initial mechanical and biological attachment was later overcome by epithelial downgrowth [267]. Despite efforts and progress, there is currently no agreement on a single strategy that provides a permanent percutaneous seal.

The need to consider the forces acting on the percutaneous implant and thereby the stresses generated in the junction between abutment and soft tissue was recognised at an early stage [112]. The stresses may lead to micro-trauma and cell activation, resulting in a constant inflammatory state of the peri-abutment soft tissue [15,36]. Accordingly, trauma at the driveline exit site is the most common initiator of late driveline exit-site infections for left ventricular assist devices, responsible for as much as 77% of the infections in one study [79]. With the recent change in the surgical technique for installing BAHS to a tissue-preservation approach, a new assumption was raised on whether the abutment can be optimised to support a relatively thick, mobile skin around the abutment.

Recently, abutments coated with hydroxyapatite (HA) were introduced clinically for BAHS [90]. The intention with this modification is to integrate the abutment material with the dermal tissue, thereby reducing the pocket formation and, as a result, inhibiting bacteria from colonising the abutment surface. Even though dermal adhesion has been demonstrated experimentally [87], the short-term clinical benefits have varied from showing no difference to showing adverse reactions compared with machined abutments [89, 90]. Four HA-coated abutments were retrieved from humans after more than 1.5 years *in situ* and the soft tissue attachment was evaluated using two-photon microscopy [268]. Tissue was found on all abutments with various amounts of tissue coverage. Biofilm, together with clinical signs of inflammation, was also detected, mostly in areas without tissue attachment. The authors concluded that evidence of skin integration was present, based on the findings of

hemidesmosomes, a basement membrane, dermal collagen and vascularisation [268].

In contrast to this approach, we hypothesised that by preventing soft-tissue adhesion and integration, the soft tissue can move more freely around the abutment, counteracting the stresses generated in the interface [Paper V]. In a situation where tissue is attached to the abutment, it is possible that chronic interfacial stresses are more challenging in comparison with a smooth abutment, which inhibits tissue attachment. Further, it has been proposed that epithelial cells prefer smoother surfaces as opposed to fibroblast cells that behave better on rougher surfaces [266, 269] although contradictory reports have been published [270]. Study V, mainly focused on bacterial sampling methodology, also evaluated the clinical outcome using a smooth, electropolished abutment for BAHS versus a standard machined abutment. However, comparable clinical outcome for both abutment topologies after three and 12 months, was revealed, using the conventional clinical outcome measures such as the Holgers score, implant stability in bone (ISQ), hygiene, pain or numbness [Paper V]. The number of patients was, however, small and the differences in surface topography and contact angle between machined and electropolished abutments were moderate (albeit statistically significant). In view of this, it might be that the effect of the limited difference in surface roughness is overruled by the overall biological events at the interface. Further studies are needed to determine the strategy, “soft-tissue integration” or “non-integration”, that is the best approach for BAHS applications.

5.3 The role of surgical technique

5.3.1 Minimally invasive approach

In Studies VI, VII and VIII, the use of a flapless minimally invasive punch technique, MIPS, for installing BAHS is evaluated. Similar to the linear incision technique for installing BAHS, raising a mucoperiosteal flap when installing a dental implant is associated with a degree of tissue damage and discomfort for the patient and it requires surgical work and suturing. Flapless surgery in dentistry was proposed to alleviate this and improve the speed and efficiency of implant placement. There are numerous advantages to this kind of approach compared with conventional flap elevation, including the preservation of soft-tissue architecture and vascular supply, reduced procedure time, no need for suturing, reduced clinical work load and the reduction of postoperative complications such as pain, swelling, infection, or dehiscence [271, 272]. Correspondingly for soft tissue reduction techniques in BAHS surgery, it has been shown that fewer postoperative complications, increased patient comfort, reduced time until loading and reduced costs are associated

with the linear incision technique compared with the more invasive dermatome and skin graft techniques [149]. Following this, a linear incision technique without any soft-tissue reduction was introduced, and it is currently the most advocated technique.

In the randomised, controlled study [Papers VII, VIII], there were more early implant losses in the MIPS group compared with the linear incision group. Even though the difference was not statistically significant, it raises concerns. At the moment, the main factors for early implant loss in association with MIPS or a linear incision approach have not been established. The main disadvantages of a flapless approach are the reduced visibility and a potential for thermal damage secondary to reduced access for external irrigation during osteotomy preparation [271]. The reduced visibility affects the surgeon's ability to visualise anatomic landmarks and vital structures and the risk of malpositioning, both when drilling and when inserting the implant, might be increased, while the depth of implant placement cannot be fully confirmed [273]. Another hypothetical drawback (suggested from dental work) of the flapless procedure is that it could interfere with osseointegration because of implant surface contamination and the deposition of epithelial and connective cells in the bone during surgical preparation [274]. Many of these drawbacks are applicable to the MIPS and BAHS situation as well. In fact, training, adherence to instructions and surgical stringency all appear to be relevant factors for success [271, 275]. The indication of a higher early extrusion rate for MIPS [Paper VIII] requires attention and should be monitored carefully to elucidate whether there is an increased risk of early implant loss using this technique compared with a flap procedure. The possible association between MIPS and overheating, flawed implant positioning or implant contamination requires further study.

5.3.2 The role of the surgical team

The flapless approach in dentistry was initially intended for novice implant surgeons; however, it soon became obvious that it requires more clinical experience, surgical judgement and pre-surgical planning than initially anticipated [271, 275]. The surgeons' technique, skills and/or judgement may influence implant survival rates. This is clearly demonstrated in a large evaluation of more than 10,000 dental implants at a single clinic where the overall, early implant failure rate ranged between 0-32% of operations performed by individual surgeons [276]. One of the main purposes of the introduction of the MIPS technique was to standardise the surgery, making it less dependent on the surgical skill of the individual surgeon [Papers VI-VIII]. With MIPS, intra-operative complications were rare and, importantly, no new intra-operative complications were identified [Paper VI]. Moreover, a reduced

surgery time was observed after training, illustrating the role of the "learning curve" [Paper VI]. Admittedly, the present thesis does not provide a clear answer as to whether the MIPS technique does indeed reduce inter-surgeon variability. Further studies are required to provide a definite answer.

5.3.3 Effects on the patient

It is also important to put the choice of surgical approach into the context of the patient. At specialised centres in the United Kingdom, the uptake rate for bone-anchored hearing aids is only about 30-40% in audiologically suitable patients with conductive hearing loss [277] and the number for single-sided deafness patients is slightly lower [278, 279]. These data are for candidates that have had a test period wearing a soft band or head band. The uptake is probably even lower at non-specialised centres without the necessary audiological expertise and infrastructure. Importantly, the main reasons for refusing BAHS implantation are patient anxiety about the surgical procedure and aesthetic concerns [277, 278]. The present thesis reveals many advantages for both the patient and the surgeon when using a minimally invasive approach, suitable for local anaesthesia. The procedure is less time consuming, bleeding is minimal, drilling and implant placement are expedited and there is no need to place and remove sutures [Papers VI, VIII]. Study VIII failed to reveal a significant difference in the incidence of adverse soft-tissue reactions between the linear incision and MIPS techniques. Nevertheless, other outcomes important to the patient, such as sensibility, cosmetics, presence of skin sagging and surgery time, were all improved for MIPS [Paper VIII]. Historically, loss of sensibility (numbness) has not been revealed as an adverse effect, as it was an inherent outcome after extensive soft tissue reduction. With preservation of the soft tissue, numbness is reduced and, with MIPS, loss of sensibility is practically eradicated [Paper VIII]. Collectively, these characteristics may prove to be important in increasing the acceptance rate for the treatment.

5.3.4 Osteotomy preparation

An implant design offering maximised initial stability would reduce the risk of implant mobility and micromotion not only in the short term but also in the long term [261]. A wider implant requires a wider drill hole, particularly if the implant surface is roughened. However, to retain a good primary stability there is a need to maximise the primary bone-to-implant contact. Secondly, thermal damage to bone cells during drilling needs to be minimised. Thirdly, the insertion torque needs to be optimised for the bone quality in question. Efficient cutting geometry of both the drills and the threaded implant is needed to balance these engineering and biomechanical requirements. This is even more important when placing implants in the hard, cortical temporal bone.

Experimental bench studies have shown that more heat is generated using surgical drill guides compared with classical implant-site preparation [280]. However, other studies did not demonstrate any difference between a flapless and an open approach [281]. Even so, fear of detrimental temperature elevation in the bone during drilling has been expressed when using a flapless or guided surgical approach compared with an open procedure [265, 271]. Based on an *in vivo* investigation in rabbit bone the consensus is that the threshold for bone necrosis, which may impede the osseointegration of an implant, is when bone is heated to a level of 47ºC [282].

One strategy in the present study [Paper IV] was to perform bench tests and compare a newly developed drill system with a clinically functioning drill system. In this case, the novel MIPS twist drill design was more effective in cutting, as demonstrated by less drilling force and increased drilling torque. Most experimental studies evaluating heat generation have been performed while mimicking standardised, uncompromised, clinical protocols, e.g. regarding irrigation, feed rate and axial speed. When performing drilling and irrigation according to standard recommended procedure it was revealed that the increase in heat generation in the surrounding artificial bone was more pronounced for the MIPS system compared with the open conventional system [Paper IV]. Nevertheless, the elevated temperatures were below the threshold mentioned above.

The consequences of deviations from the standard protocol have been less investigated. In the present bench test, two important results were obtained. Firstly, a reduction in irrigation significantly increased heat generation, irrespective of drill system. Secondly, a striking finding was the sensitivity of the MIPS system to a prolonged drilling sequence (the drill was left spinning after reaching the full depth). In this situation, the mean peak temperature increase was about 2.5 times higher for MIPS compared with the conventional system, despite full irrigation [Paper IV]. In a recent experimental study, measuring heat generation when drilling *ex vivo* in bone and artificial bone, it was suggested that the main heat source was not plastic deformation and the shearing of the material at the cutting point but rather the friction caused by the chip debris travelling through the drill flutes [283]. This could, at least partly, explain the difference after prolonged drilling between the MIPS and the conventional system. Although definite proof is not provided in this study, for MIPS, one assumption is that the cannula blocks the evacuation of the heated chips from the osteotomy site. In contrast, the open procedure might allow for a more efficient exchange of chips and cooling fluid. Taken together, the importance of performing a drilling procedure according to instructions is demonstrated, with respect to both irrigation and adherence to drilling instructions.

Most studies in this field are performed in different bench tests, showing the importance of a proper drilling protocol [283, 284]. However, *in vivo*, additional factors such as bone quality, vascularisation and other host-site characteristics are important. For example, the critical role of osteocytes in detecting and initiating the remodelling cascade in neighbouring cells in response to thermal damage is difficult to simulate in bench tests [285]. More detailed research is needed to clarify the extent to which non-optimal drilling sequences or irrigation influence cell injury, regenerative processes and implant survival.

5.4 The role of host defence and bacteria

Nearly 40 years after the seminal works on design criteria and failure modes for percutaneous implants by von Recum, Grosse-Siestrup, Hall and co-workers [3, 85, 112], a reliable system still remains to be developed. The Achilles heel of the percutaneous access is the junction between tissue, abutment and the external environment. This is exemplified by the ventricular assist devices, a highly complex and technologically advanced life-support device, where the percutaneous driveline is the most common site of infection, occurring in up to half of the patients [82, 93].

The skin is breached when a percutaneous implant is installed, leading to a local disruption of homeostasis. Due to this breach, the host defence system is upregulated. This is demonstrated by the chronic presence of inflammatory cells close to the abutment, even in cases without macroscopic signs of inflammation [58, 286]. The relationship between the number of bacteria, their virulence and the local and systemic host defence system is largely unknown. For percutaneous devices, *S. epidermidis* and *S. aureus* are regarded as the main pathogens [113, 287, 288]. This was further confirmed in the abutment study [Paper V], where *S. aureus* was identified in half the patients and *S. epidermidis* was found in all but one patient.

For a percutaneous system, there are basically seven compartments in which bacteria can be present; (i) in the blood, (ii) on the skin, (iii) in the surrounding soft tissue, (iv) in bone, (v) on the fixture, (vi) on the abutment surface and finally, (vii) in the peri-abutment space. The bacterial colonisation of any of these sites may eventually lead to superficial or deep infection. The bacteria may reside either in the extracellular matrix or intracellularly [91, 289]. In Study V, one aim was to sample, identify and quantify colonising bacteria in three of the compartments mentioned above; on the retrieved abutment, in the peri-abutment exudate and in the peri-abutment soft tissue [Paper V]. In the retro-auricular position where a BAHS is installed, no aerobic bacteria were detected in the soft tissue (after disinfection of the skin surface), whereas

anaerobes were found. In contrast, after the installation of the percutaneous BAHS, both aerobic and anaerobic bacteria were detected in all three compartments at both three and 12 months. These observations indicate that the surgery, installation and presence of a percutaneous implant alter the microbiological flora. Interestingly, in five out of 12 patients, the same bacterial species (with an identical biochemical numerical profile) were found at both three and 12 months. Moreover, the same species was found in at least two of the three compartments at the same time point. Taken together, the sampling and analytical approach appear suitable for further studies on abutment modifications. Although it was beyond the aims of Study V, to answer why infection occurs at BAHS, several questions can be formulated based on the present results.

- Is there a specific bacterial flora composition associated with an increased risk of infection?
- Is the onset of infection caused by a change in the virulence properties of the colonizing "commensal" bacteria, or by newly arriving pathogens?
- What is the minimal dose of a specific pathogen to cause clinical signs of infection at BAHS?

Against the background of the work by Holgers and co-workers [58, 59, 286], showing an elevated number of inflammatory and immune cells in association with the abutment, ongoing studies are also aiming to determine the molecular changes in the tissue cells retrieved from the three evaluated compartments.

Taking strictly bone-anchored percutaneous implants into consideration, a comparison between BAHS and osseointegrated implants for limb prosthesis attachment is of interest. There are important similarities, as well as differences, between these two types of osseointegrated percutaneous implant. For many years, the results for the treatment of amputees were discouraging, with biomechanical problems and infection. Today, several systems are in clinical use, including the pioneering so-called OPRA system [290, 291], providing improved long-term, patient-reported outcomes compared with the conventional orthotic treatment. In spite of this, for bone-anchored femoral amputation prostheses, the 10-year cumulative risks of osteomyelitis (deep infection) and implant extraction due to infection are 20% and 9%, respectively [97]. These figures are higher than those reported for BAHS [99, 151]. Although the bacterial flora varies on the body [61], for both devices, *S. epidermidis* and *S. aureus* are either the main colonisers of the percutaneous entrance or pathogens implicated in deep infections [116, 287, 288]. Nevertheless, species like *Enterococcus faecalis* have been identified for transfemoral amputation implants, especially for a shorter length of the

amputated femur [116] whereas *Propionibacterium acnes* is present for BAHS [288]. In comparison with BAHS, a higher frequency of inflammatory signs (Holgers score) was found in the peri-abutment skin of bone-anchored femoral amputation prostheses [116]. Possible reasons for the differences in infection risk and inflammation between these two applications are differences in underlying disease, skin and bone properties, the uptake of mechanical load, relative movements between tissue and implant, the size and macroscopic design of the systems and the cleaning regimen.

Strategies to reduce infection in a specific application require an in-depth understanding of all the aspects that influence the performance of a percutaneous implant system. These aspects include the selection of patients, the host site and its status, the configuration of the implant system, the surgery, the healing, the maintenance, the site's microbial load and type, and the treatment of adverse reactions. With respect to the implant system and its relationship to tissues, the concept of "winning the race for the surface" by host cells [72] has been advocated as an important principle. In recent years, a number of basic design criteria for implants have been suggested in order to reduce the risk of infection [92]. There are, however, many challenges in bringing new infection-resistant, antimicrobial biomaterials and coatings for implants and devices to the patients. Challenges in terms of both valid experimental models that simultaneously evaluate tissue integration, bacterial colonisation and immune responses and the difficulty translating promising technologies to reasonable clinical trials and subsequent human use.

5.5 Maintenance of the exit site

The structural and functional barrier of the skin is affected by a percutaneous material and temporary and permanent percutaneous devices have high infection rates (Table 1). It is therefore logical to address the role of the skin condition and the effects of skin and implant-skin interface maintenance regimes for such serious complications. The present thesis only briefly addresses this important issue. In Study V, it was hypothesised that a polished abutment surface, by virtue of its smoothness and ease of cleaning, would reduce microbial colonisation and debris. However, an improved outcome using an electropolished abutment could not be demonstrated using the conventional macroscopic clinical outcome measurements. Even if there was a weak correlation between hygiene and the Holgers score, caution extrapolating this finding is advised. First, hygiene was subjectively scored and defined as "amount of debris around the abutment" and, secondly, few patients were included in the study.

Metal pins are used to apply skeletal traction or external fixation devices in the management of orthopaedic fractures. The post-operative pin care protocol often includes cleaning, dressing changes and showering. The same applies to BAHS, with the exception that a dressing is only applied during the first post-operative week. Despite several studies to provide evidence-based recommendations for pin-site care and regimens for the prevention of pin-site infections, substantial controversy exists with regard to the optimal protocol [113]. A Cochrane Systematic Review was not able to determine an optimal strategy for pin-site care based on the available literature [83]. Although randomised, most studies were small and underpowered with methodological flaws, and none was blinded. Minimal efforts were made to control for other factors that might have influenced the results, such as patient compliance, antibiotic use, location of pins, pin-insertion procedures, types of hardware used, variation in the age of participants and condition being treated by external fixators [83].

The importance of cleaning was also apparent in the dog studies with the stoma implant [Paper II]. After applying a cleaning regime with twice-daily cleaning and flushing of the skin area with soap and water, the skin status, at least macroscopically, improved.

This illustrates the challenges associated with generating a common maintenance and treatment regimen for a percutaneous application. To the author's, knowledge, no studies have been performed for BAHS on any of these topics. Moreover, the manufacturer's instructions are very scarce and clinics commonly rely on their own experience.

5.6 Assessment and outcome measures

To be able to judge the clinical effects of an implant system or surgical technique, precise assessments and outcome measures are needed. Historically, a great deal of interest has focused on the status of the soft tissue around the exit site. The Holgers score has been extensively used since its introduction in the late 1980s [115]. The Holgers score is determined solely based on observations made by health-care professionals. It consists of serial observations regarding severity with a dichotomous outcome (present/not present). The scale can be used to indicate treatment, for example, topical treatment for Holgers grade 2 or revision surgery for Holgers grade 4; however, these treatment decisions are not standardised worldwide. It is important to acknowledge that the Holgers score, and other modified scales [116] are a macroscopic assessment of the characteristics and appearance of the skin adjacent to the percutaneous abutment. The Holgers score therefore measures

the local inflammation and repair, rather than being a quantification of infection.

The advantages of the currently used Holgers score are its ordinal scale and overall simplicity that result in its high usability. In addition to being subject to personal interpretations in indicating treatment, the Holgers score has some limitations. First, the current scale was originally developed when evaluating the skin three months after implantation. It therefore lacks the ability to describe complications in wound healing, such as dehiscence. Secondly, the formation of a granulation ring but not the skin height is incorporated in the Holgers score. The skin height parameter is important, as excessive soft-tissue growth can encircle the abutment without signs of inflammation or infection. In turn, this may result in the inability to couple the sound processor, requiring abutment change or skin revision. Thirdly, the Holgers score lacks an assessment of pain. Pain can result from skin infection, but there are also examples of unexplained chronic pain eventually leading to the elective removal of the implant [224]. The early failure of osseointegration has been shown to be associated with a high and sustained perception of pain prior to the loss of an implant [33]. For BAHS, we found a positive correlation between the Holgers score at three months and the reported pain score at the same time point [Paper V], suggesting an association between the degree of inflammation and pain.

Any scale or instruction should be easy to implement and practical to use and evaluate. The scale should preferably be (i) true, i.e. the scale measures what it intends to, (ii) discriminatory, i.e. the outcome measure distinguishes a change between groups and is reliable in test-retest and (iii) feasible, i.e. the outcome measure should be easy to implement in different healthcare settings without great expense in terms of time and money [117]. Further, it should preferably be derived from the perspective of the patient rather than that of the clinician (patient-centred outcome measure). In connection to the recent proposed outcome scale for bone-conduction hearing implants, the IPS-scale, a standardised treatment advice for each IPS-scale is proposed [120]. Future studies are needed to determine the reliability and biological validity of both the Holgers score and IPS-scale.

The use of biomarkers provides a means of disclosing positive or negative responses to a specific treatment or intervention. These techniques have been introduced to increase our understanding of the interfacial events for skin-penetrating devices [116, 292]. The non-invasive sampling of the peri-abutment fluid for the subsequent calculation of the relative gene expression of selected cytokines using quantitative polymerase chain reaction (qPCR) is of particular interest. These data demonstrate that peri-abutment tissues

showing clinical signs of inflammation or showing the presence of bacteria are associated with increased fluid exudate, which contains elevated levels of key biomarkers of inflammation around BAHS [116, 292].

It is concluded that systematic work on post-operative outcome and maintenance measures, involving all stakeholders, is encouraged.

5.7 Limitations and advantages

Several limitations are of concern in this thesis. The studies investigating the opportunity to obtain integration between the bowel and a percutaneous implant with the aim of developing a continent stoma [Paper I, II] were exploratory and developmental in nature. As a result, the implant design, the surgical model and post-operative maintenance underwent changes throughout the studies. On one hand, this was a strength as a functional model could be created. On the other hand, strict comparisons between models were difficult to perform. One main limitation of the models used was the movements of animals, preventing undisturbed healing. Further, no microbiological, immunological or molecular techniques were employed to elucidate the responses in the regions of interest.

Study III aimed to elucidate the biomechanical and morphological correlates of osseointegration. It was strengthened by the fact that the only difference between test and control was the surface modification [Paper III]. However, only one time-point was considered (eight weeks) and no emphasis was placed on the molecular mechanisms which could be regarded as limitations of the work. On the other hand, previous studies by the research group have shown that the selected observation period is an optimal time point in this model for determining whether osseointegration has developed. This can also be regarded as a way of reducing the number of animals. From a scientific perspective, a decision was made to deepen our morphological and ultrastructural understanding of the implant-bone interface (using BSE-SEM, TEM and STEM) which is of importance for the interpretation of the biomechanical properties of the implant-bone interface. The use of molecular techniques would require additional animals and observation periods in order to be meaningful.

One main advantage of the evaluation of heat generation during the preparation of an osteotomy [Paper IV] is the panel of drilling procedures evaluated, the number of drillings executed for each case and using four probes for each osteotomy. In addition, since thermocouple measurements are highly sensitive to the distance to the heat source, the scanning of the blocks enabled the exact determination of the position of each thermocouple. The drill system used as

control is successfully used in the clinic, which considerably strengthens the relevance of the comparison between test and control. On the other hand, the actual biological consequences of a specific temperature increase in viable bone is unknown at this stage, as the measurements were performed in artificial composite material. Although a small number of histological evaluations were made in bovine cadaver bone, further studies on the effects of the drill systems in viable bone are needed.

To our knowledge, this is the first time in the field of BAHS where the microbiological status in the tissue, in the peri-abutment space and on the abutment has been evaluated over time [Paper V]. This pilot trial demonstrated the opportunity for bacteriological sampling from several compartments in forthcoming clinical studies. The sample size was too small to be able to advocate that one specific compartment is more important than another. The interesting relationships between clinical outcomes and microbiological findings should preferably be strengthened in larger patient cohorts.

The introduction of a new surgical technique involves learning curves and possibly also teething trouble. The aim of the service evaluation of MIPS [Paper VI] was therefore to evaluate MIPS across multiple centres and surgeons with the emphasis on intra-surgical events and short-term outcome. As such, it lacks a more complete set of outcome measurements, as the evaluation relies on the standard method of collecting data in individual centres rather than adhering to a single study protocol. Further, one obvious weakness of the current evaluation is the lack of long-term follow-up.

It would have been preferable for the randomised, controlled trial [Papers VII, VIII] to have been blinded. Due to practical considerations, this was not the case here. In future studies, blinded, randomised, controlled trials could be attempted when comparing punch-only techniques, implants or abutments.

6 SUMMARY AND CONCLUSIONS

Percutaneous implants are medical devices crossing the epithelial barrier through a surgically created defect, thus permitting access to the interior of the body. Irrespective of anatomical site and clinical application, there are multiple challenges to such implants, in particular if long-term function is required.

In the present thesis, the tissue response and clinical outcome to systematic changes of device design, drill system and drilling procedure, and surgical procedure were investigated. A percutaneous stoma port was evaluated experimentally whereas a percutaneous bone-anchored hearing system (BAHS) was evaluated experimentally and clinically using a combination of morphological, spectroscopic, microbiological and biomechanical techniques, and clinical outcome measures.

– Experimental studies demonstrate the opportunity to integrate a soft-tissue-anchored titanium implant with visceral tissues. However, the integration is challenged by the presence of bacteria and mechanical strains between the implant and the soft tissue. Unless barriers at both implant-skin and implant-intestine junctions are created, implant performance is jeopardised [Papers I, II].

– A micro- and nano-scale laser modification of a screw-shaped titanium implant exhibits bonding to bone, improved stability and a different load deformation pattern compared with a machined surface. In the newly formed bone within the threads, the extracellular matrix, the bone area, bone contact and osteocyte densities are comparable between the machined and laser-modified implants. There is a strong correlation between removal torque and the degree of bone-to-implant contact as well as between resonance frequency analysis at retrieval and the bone area [Paper III].

– During osteotomy preparation, bench tests in artificial bone demonstrate that the level and distribution of heat surrounding the osteotomy are significantly affected by the drill design, drilling procedure and effectiveness of irrigation. Provided the clinically recommended drilling procedure is adhered to, the absolute temperatures using either a conventional drill system or a guided drill system are below the threshold for thermally induced tissue damage. Whereas a guided drilling approach generates relatively more heat compared with a conventional system, especially during a prolonged drilling sequence, it is more forgiving in the case of impaired irrigation. Furthermore, the results indicate that a

twist drill design is more efficient and less tissue damaging compared with a conventional drill design [Paper IV].

– Already at baseline, prior to installing a bone-anchored hearing system, the soft tissue at the retro-auricular position contains mainly anaerobic bacteria. During the one-year follow-up, *S. epidermidis* is present at the implant site in most patients whereas *S. aureus* is present in half the patients. The clinical outcome during the first year after BAHS installation is similar for machined and polished abutments. For polished abutments, more bacteria are detected on the abutment surface and in the peri-abutment compartment compared with machined abutments. Several associations between clinical parameters and the type and number of micro-organisms warrant further studies using larger patient cohorts [Paper V].

– A minimally invasive technique (MIPS) for installing BAHS results in a short surgery time and few intra-operative complications. Good soft-tissue outcome and implant survival are demonstrated in the short term [Paper VI].

– A multicentre, randomised, controlled trial is designed to compare the minimally invasive technique (MIPS) with the linear incision technique with soft-tissue preservation [Paper VII].

– At three months, skin sensibility, cosmetics, the presence of skin sagging and surgery time are significantly better for the MIPS group compared with the linear incision group. The incidence of inflammation, dehiscence, pain and soft-tissue overgrowth is similar for the groups [Paper VIII].

In conclusion, the work presented in this thesis demonstrates that implant configuration and design, surgical approach and drilling procedure, as well as host site, are all important factors influencing the tissue response to percutaneous implant systems.

7 FUTURE PERSPECTIVES

The findings reported in this thesis shows that the performance of a skin-penetrating implant is related to multiple interrelated factors. Using bench tests, animal studies and clinical studies together with employing a variety of analytical techniques, some of these factors have been investigated and determined.

In the context of bone-anchored percutaneous implants and specifically BAHS, it would be pertinent to further explore:

- The relationship between clinical, microbiological and molecular outcomes following BAHS surgery in a larger cohort of patients to further understand the key determinants of the soft tissue outcomes.
- The effect, in terms of implant survival, of the clinical use of laser-modified implants, specifically in children and in patients with compromised conditions.
- The effect of aftercare maintenance regimes using a systematic approach.
- To collect and analyse clinical explants by implementing a systematic retrieval protocol, efficient preservation of the tissue for different state-of-the-art analytical techniques and by a logistic scheme. Thereby being able to correlate the clinical history of patients with failed implants with the underpinning microbiological, cell biological and morphological finger-prints achieved in the laboratory.

In the context of osteotomy preparation, it would be of interest:

- To better understand the deformation and fracture processes associated with osteotomy machining.
- To visualize the distribution of irrigant during cutting and correlate these data with temperature measurements.
- To clarify to what extent a specific drilling protocol will influence cell injury, regenerative processes and implant survival *in vivo*.

For a continent enterostoma implant it would be of interest to explore:

- Implant designs based on tissue-engineered constructs.
- Infection-resistant multifunctional (contact killing and releasing) coatings in combination with a 3-stage surgical procedure.

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